Alternative treatments in attention deficit hyperactivity disorder

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ALTERNATIVE TREATMENTS IN ATTENTION DEFICIT HYPERACTIVITY DISORDER

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This thesis is dedicated to my father Kurt, who sparked my interest in science and in so many ways provided me with the motivation to research new and better treatments for those who experience psychological distress.

Abstract

Attention-deficit/hyperactivity disorder (ADHD) is characterised by clinically impairing levels of inattention and hyperactivity/impulsivity along with cognitive deficits and problems with emotional lability (EL). Stimulant medication, although the first line treatment, can be problematic due to for example, adverse effects and partial response. Patients often explore alternative treatments.

In children, a small to moderate effect of Omega-3 polyunsaturated fatty acid supplementation (*n*-3 PUFA) in reducing ADHD symptoms has been found. However effects on cognition and EL are unclear. Part one, consists of two meta-analyses examining the effects of *n*-3 PUFA supplementation on cognition and EL. In children with ADHD or with a related neurodevelopmental disorder, there was suggestive evidence for effects on EL with little evidence for effects on cognition. In the general population there was little evidence for effects on cognition. A randomised-controlled trial (RCT) of n-3 PUFA supplementation has not yet been conducted in adults with ADHD.

Part two, was an RCT of *n*-3 PUFA supplementation in 81 adults with ADHD. Baseline case/control comparisons showed the ADHD cases to have impaired cognitive performance and high levels of EL, with no difference in n-3 PUFA levels. Supplementation with n-3 PUFA in the ADHD cases indicated no beneficial effect in the intent-to-treat analysis but some indication of an effect in the per-protocol analysis.

Self-medication with cannabis appears common in ADHD. Part three was an RCT of the cannabinoid medication, Sativex in 30 adults with ADHD. Results indicated a beneficial effect on ADHD symptoms and potentially cognitive performance and EL.

The cannabinoid medication, Sativex, could be a promising avenue for further research as an alternative treatment for adults with ADHD. Although evidence for n-3 PUFA supplementation is weaker, it could have a small to moderate effect in adults with ADHD, further research is warranted.

Statement of work

Chapters 2 and 3: The proposals for the meta-analyses were conceived by myself and my supervisor Professor Philip Asherson. Expert advice was provided by Dr Evangelos Vassos, Dr Jonna Kuntsi and Dr Charlotte Tye. I carried out all aspects of the two projects: the literature search, quality assessment of studies (second rated by Dr Charlotte Tye), data analysis (closely supervised by Dr Vassos), interpretation and write-up of the two projects.

Chapter 5: The project idea, proposal, funding application and ethical approval for the OCEAN Study were carried out by Professor Philip Asherson and Ms Lena Johansson. I was responsible for recruitment, data collection, supervision and training of research staff working on the project, and was responsible for sourcing and preparing all the equipment and tasks used in this project. I carried out day-to-day project coordination over approximately 2 years, and was a key contributor to recruitment, participant selection, the organisation and carrying out of the assessments and the management of the data. All analyses were carried out by myself, supervised by Seth Seegobin and Professor Asherson.

Chapter 6: The EMA-C project was conceived through discussions between myself and Professor Asherson. The proposal, application to GW Pharma for provision of the cannabinoid (Sativex) and placebo medication and ethical approval for the EMA-C study was completed by myself supervised by Professor Asherson. I was responsible for the recruitment, supervision and training of the staff member (Emma Williams (EW)) working on the project and was responsible for sourcing and preparing all the equipment and tasks used in this project. I am extremely grateful to Ms Williams who completed the data collection and management. The selection of participants was completed by EW, myself and Prof Asherson. The monitoring of participants during the titration period and trial (regular phone calls) was completed by myself. I am hugely grateful to Prof Asherson who advised on all medical queries. All analyses were carried out by myself supervised by Seth Seegobin and Professor Asherson.

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Publications and presentations relevant to this thesis

Publications

Chapter 2 is:

Cooper, R.E., Tye, C., Kuntsi, J., Vassos, E., Asherson, P., (2015). Omega-3 polyunsaturated fatty acid supplementation and cognition: A systematic review and meta-analysis. Journal of Psychopharmacology. 29(7), 753–763

Chapter 3 is adapted from:

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Presentations

Cooper, R.E., Tye, C., Kuntsi, J., Vassos, E., Asherson, P. Omega-3 essential fatty acids in ADHD. Fourth Congress of the UK Adult ADHD Network (UKAAN) "Mind, Body & Brain", 10-12 September 2014, London, UK (Talk).

Cooper, R.E., Kuntsi, J., Tye, C., Asherson, P. An objective measure of emotional lability in adults with ADHD. Eunethydis International Conference on ADHD, 21-24 May 2014, Istanbul, Turkey (poster presentation).

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Conflict of interest

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Abbreviations

Abbreviation	Meaning
AAD-UK	Adult Attention Deficit Disorder site: http://aadduk.org/about/
AAQoL	Adult ADHD Quality of Life Scales
ADDISS	National Attention Deficit Disorder Information and Support Service – website: http://www.addiss.co.uk/
ADHD	Attention Deficit Hyperactivity Disorder
ADHD+RD	Attention Deficit Hyperactivity Disorder or Related Disorder
ADHD+RND	Attention Deficit Hyperactivity Disorder or Related Neurodevelopmental Disorders
AE	Adverse Events
ALA	Alpha Linoleic Acid
ALS	Affective Lability Scale
ALS-SF	Affective Lability Scale-Short Form
ASD	Autism Spectrum Disorder
ASRS	Adult Self Rating Scale for ADHD
BLEQ	Brief Life Events Questionnaire
BRIEF-A	Behaviour Rating Inventory of Executive Function – Adult Version
CAADID	Conners' Adult ADHD Diagnostic Interview for DSM-IV
CAARS	Conners' Adult ADHD Rating Scales
CAS	Children's Aggression Scale
CB1R	Cannabinoid Receptor Type 1
CBCL	Child Behaviour Checklist
CBD	Cannabidiol
CBT	Cognitive Behavioural Therapy
CCQ	Cognitive Control Questionnaire
CE	Commission Errors
CI	Confidence Intervals
CIAO	Concerta In Adult ADHD
CNS-LS	The Centre for Neurological Study Lability Scale
CNVs	Copy Number Variants
CONSORT	Consolidated Standards of Reporting Trials
CPT	Continuous Performance Test
CPT-OX	Cued Continuous Performance Test
CTIMP	Clinical Trial of an Investigational Medical Product
CV	Coefficient of Variation
DA	Dopamine
DAT	Dopamine Transporter
DCD	Developmental Coordination Disorder
DHA	Docosahexaenoic Acid
DIVA	Diagnostic Interview for ADHD in Adults
DMN	Default Mode Network
DPA	Docosapentaenoic Acid
DSM-5	Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders
DSM-III	Third Edition of the Diagnostic and Statistical Manual of Mental Disorders
DSM-IV	Fourth Edition of the Diagnostic and Statistical Manual of Mental Disorders
DTQ	Depressive Thoughts Questionnaire
ECS	Endogenous Cannabinoid System
EEG	Electroencephalography
EL	Emotional Lability

EMA-C	Experimental Medicine in ADHD - Cannabinoids
EPA	Eicosapentaenoic Acid
ES	Effect Size
FCS	Fully Conditional Specification
FDA	United States Food and Drug Administration
FEBA	Female Experiences and Brain Activity
FFQ	Fish Frequency Questionnaire
GLA	Gamma-Linoleic Acid
GWAS	Genome Wide Association Studies
HC	High Concentrated
ID	Identification
IoPPN	Institute of Psychiatry Psychology and Neuroscience
IQ	Intelligence Quotient
ISI	Inter-Stimulus Interval
ITT	Intent to Treat
KCL	Kings College London
LS-MEANS	Least-Square Means
MAR	Missing At Random
MHRA	Medicines and Healthcare products Regulatory Agency
MI	Multiple Imputation
MINI	Mini International Neuropsychiatric Interview
MNAR	Missing Not At Random
MOAS	Modified Overt Aggression Scale
MRC	Medical Research Council
MRT	Mean Reaction Time
MS	Multiple Sclerosis
N	Number
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
Non-CTIMPS	Non-Clinical Trials of an Investigational Medical Product
NRES	National Research Ethics Committee
OCD	Obsessive Compulsive Disorder
OCEAN	Oils and Cognitive Effects in Adult ADHD Neurodevelopment
OE	Omission Errors
PASAT-C	The Computerized Paced Auditory Serial Addition Task
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSQI	Pittsburgh Sleep Quality Index
PUFA	Polyunsaturated Fatty Acids
QbTest	Quantitative Behaviour Test
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RDs	Related Disorders
RTV	Reaction Time Variability
SART	Sustained Attention to Response Task
SAS	Statistical Analysis Software
SCL-90	Symptom Checklist-90
SD	Standard Deviation
SDRT	Standard Deviation of Reaction Time
SE	Standard Error
SES	Socio-economic Status
SGDP	Social, Genetic and Developmental Psychiatry Centre

SLaM	South London and Maudsley Hospital
SMD	Standardised Mean Difference
SPECT	Single Photon Emission Computed Tomography
SUD	Substance Use Disorder
TD	Typically Developing
THC	Tetrahydrocannabinol
TOVA	Test of Variables of Attention
UKAAN	UK Adult ADHD Network
VLF-EEG	Very Low Frequency Electroencephalography
WASI-II	The Weschler Abbreviated Scale of Intelligence II
WFIRS-S	Weiss Functional Impairment Rating Scale Self Report
WIAT-II	Wechsler Individual Achievement Test II
WISC	Wechsler Intelligence Scale for Children
WRAADS	Wender-Reimherr Adult Attention Deficit Disorder Scale
WRAT	Wide Range Achievement Test
Δ9-THC	Δ9-tetrahydrocannabinol

Chapter 1: Introduction

1.1 Overview of Attention-deficit/hyperactivity disorder (ADHD)

1.1.1 Historical background

In 1798 one of the first known accounts of what we now call Attention Deficit Hyperactivity Disorder (ADHD) was recorded by Alexander Crichton, describing a state of 'mental restlessness' (Crichton, 2008). At the start of the twentieth century a series of case studies were reported by George Frederick Still (considered the father of British paediatrics) in the prestigious Goulstonian lectures (Still, 2006). Treatment attempts with amphetamines were described as early as 1937 by Bradley who later, in a seminal paper, described the effects of stimulant medication in groups of children including those who would be categorised as having ADHD (Bradley, 1950). In the 1940s the brain was suggested to be the source of ADHD-like symptoms which were described as minimal brain damage in the wake of an encephalitis epidemic (Lange, Reichl, Lange, Tucha, & Tucha, 2010). ADHD in adulthood was first formally described in the 70s with the successful treatment of adult patients exhibiting symptoms of ADHD, with stimulant medication (Wood, Reimherr, Wender, & Johnson, 1976). In 1980, the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) created the first reliable operational diagnostic criteria for the disorder. The establishment of criteria started numerous programmes of research which resulted in the scientific community viewing ADHD as an impairing, often persistent, developmental and neurobiologically-based disorder of high prevalence caused by a complex interplay of genetic and environmental risk factors.

1.1.2 Diagnostic classification

ADHD is characterised by the core behavioural symptoms of impulsivity, hyperactivity and/or inattention. Classified as a neurodevelopmental condition, it emerges in childhood and persists into adulthood and has both biological and environmental underpinnings (Biederman, Petty, Clarke, Lomedico, & Faraone, 2011; Bolea-Alamañac et al., 2014; Gittelman, Mannuzza, Shenker, & Bonagura, 1985; Greenfield, Hechtman, & Weiss, 1988; Ronald Kessler, Adler, Barkley, et al., 2005; Lara et al., 2009; Mannuzza et al., 1991; Wenwei, 1996).

The recent publication of the fifth edition of the DSM (The DSM-5) acknowledges the continuation of ADHD into adulthood and provides several changes from the previous DSM-IV: the ADHD

diagnosis in adults can be made with five instead of the six symptoms required in childhood; concurrent diagnosis of ADHD and Autism Spectrum Disorder (ASD) can now be made; and childhood symptoms (without impairment) can be present before 12 years instead of 7. The DSM-5 criteria for ADHD is detailed below (American Psychiatric Association, 2013).

DSM-5 criteria for ADHD

People with ADHD show a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development:

Inattention: Six or more symptoms of inattention for children up to age 16, or five or more for adolescents 17 and older and adults; symptoms of inattention have been present for at least 6 months, and they are inappropriate for developmental level:

- Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or with other activities.
- Often has trouble holding attention on tasks or play activities.
- Often does not seem to listen when spoken to directly.
- Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., loses focus, side-tracked).
- Often has trouble organizing tasks and activities.
- Often avoids, dislikes, or is reluctant to do tasks that require mental effort over a long period of time (such as schoolwork or homework).
- Often loses things necessary for tasks and activities (e.g. school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones).
- Is often easily distracted
- Is often forgetful in daily activities.

Hyperactivity and Impulsivity: Six or more symptoms of hyperactivity-impulsivity for children up to age 16, or five or more for adolescents 17 and older and adults; symptoms of hyperactivity-impulsivity have been present for at least 6 months to an extent that is disruptive and inappropriate for the person's developmental level:

- Often fidgets with or taps hands or feet, or squirms in seat.
- Often leaves seat in situations when remaining seated is expected.

- Often runs about or climbs in situations where it is not appropriate (adolescents or adults may be limited to feeling restless).
- Often unable to play or take part in leisure activities quietly.
- Is often "on the go" acting as if "driven by a motor".
- Often talks excessively.
- Often blurts out an answer before a question has been completed.
- Often has trouble waiting his/her turn.
- Often interrupts or intrudes on others (e.g., butts into conversations or games).

In addition, the following conditions must be met:

- Several inattentive or hyperactive-impulsive symptoms were present before age 12 years.
- Several symptoms are present in two or more settings, (e.g., at home, school or work; with friends or relatives; in other activities).
- There is clear evidence that the symptoms interfere with, or reduce the quality of, social, school, or work functioning.
- The symptoms do not happen only during the course of schizophrenia or another psychotic disorder. The symptoms are not better explained by another mental disorder (e.g. Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder).

Based on the types of symptoms, three kinds (presentations) of ADHD can occur:

***Combined Presentation*:** if enough symptoms of both inattention and hyperactivity-impulsivity were present for the past 6 months.

***Predominantly Inattentive Presentation*:** if enough symptoms of inattention, but not hyperactivity-impulsivity, were present for the past six months.

***Predominantly Hyperactive-Impulsive Presentation*:** if enough symptoms of hyperactivity-impulsivity but not inattention were present for the past six months.

***In partial remission**:* DSM-5 also classifies sub-threshold cases as 'in partial remission' cases when full criteria were previously met and current subthreshold symptoms still lead to impairments.

1.1.3 Emotional lability

The recent DSM-5 lists emotional lability (EL), characterised by irritability, mood lability and low frustration tolerance, as a characteristic feature of ADHD that may be used to support the diagnosis (American Psychiatric Association, 2013). EL is associated with persistence of ADHD into adulthood and is independently associated with a wide range of occupational, social and educational impairments (Anastopoulos et al., 2011; Barkley & Fischer, 2010; Skirrow & Asherson, 2012). Therefore EL is associated with increased impairment in ADHD.

Debate as to whether EL reflects a core domain of ADHD in adults is ongoing. Arguments in favour point toward the common co-occurrence of EL in adults with ADHD (Barkley & Fischer, 2010; Barkley & Murphy, 2010) which remains in a non-comorbid sample (Skirrow & Asherson, 2012); evidence that treatment with ADHD medication (e.g. methylphenidate or atomoxetine) has a similar effect size on reducing the core symptoms of inattention or hyperactivity/impulsivity as it does on EL (Reimherr et al., 2005, 2007; Rosler et al., 2010; reviewed in Skirrow, McLoughlin, Kuntsi, & Asherson, 2009); that EL is present at an increased rate in family members of individuals with ADHD (Epstein et al., 2000; Surman et al., 2011) and shares genetic influences with ADHD (Merwood et al., 2014). However, against the notion of EL as a core ADHD domain is that it is non-specific, occurring across a range of other psychiatric and neurodevelopmental disorders. For example, irritability and temper problems are often included in the diagnostic criteria for borderline personality disorder and bipolar disorder (American Psychiatric Association, 2013; Asherson et al., 2014). As will be discussed in section 1.1.5. such comorbidities are frequent in ADHD with mood disorders as high as 38% in adults (Kessler et al., 2006) and around 60% of children may have oppositional defiant disorder (Kadesjö & Gillberg, 2001). Comorbidity has also been shown to be associated with severity of EL in ADHD children and adolescents (Anastopoulos et al., 2011; Sobanski et al., 2010). Therefore although the nature of the relationship of EL in ADHD is still debated, it is recommended that individuals with chronic problems with emotion regulation or mood instability should always be screened for ADHD (Asherson et al., 2014).

1.1.4 Prevalence

Meta-analyses of over 100 studies have estimated the worldwide prevalence of ADHD in children and adolescents to be around 5% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007;

Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014). This estimate was subject to significant variability due to the choice of diagnostic criteria, the source of information used, and the inclusion of the requirement for functional impairment, as well as symptoms, for diagnosis. Prevalence rates did not differ widely by geographical location. In addition there was no evidence worldwide to suggest an increased prevalence of ADHD over the past three decades (Polanczyk et al., 2014).

Meta-analyses suggest the pooled prevalence of ADHD in adulthood is around 2.5% (Simon, Czobor, Bálint, Mészáros, & Bitter, 2009). In older adults a similar prevalence rate (2.8%) has been found (Michielsen et al., 2012). Longitudinal follow-up studies of children with ADHD find that approximately two-thirds of youths with ADHD retain impairing symptoms of the disorder (i.e. they meet partial diagnostic status with impairments) by the age of 25 years (Faraone, Biederman, & Mick, 2006). Interestingly, in a follow-up study (at a mean age of 11 and 18 years) of children with ADHD (in the UK) a much higher persistence rate of around 80% was found for the full diagnosis. This may have been due to the selection of DSM-IV combined subtype cases at baseline (Cheung et al., 2015). Similar unpublished findings were found by the Dutch group led by Jan Buitelaar.

In children and adolescents ADHD predominantly affects males with a male to female sex ratio in the order of 4:1 in clinical studies and 2.4:1 in population studies (Polanczyk et al., 2007). However epidemiological and clinical studies indicate the sex discrepancy almost disappears in adulthood (Kooij et al., 2005; Kooij, Aeckerlin, & Buitelaar, 2001; Matte et al., 2015), potentially due to a referral bias amongst treatment-seeking patients, or potentially sex-specific effects of ADHD over the course of the disorder (Faraone et al., 2015). Although some studies have associated ethnicity with the ADHD diagnosis (Lingineni et al., 2012; Visser et al., 2014), this may be due to referral patterns and barriers to care that disproportionately affect particular ethnic groups. It has therefore been concluded that the true prevalence of ADHD does not vary with ethnicity (Faraone et al., 2015).

1.1.5 Comorbidity

ADHD in both childhood and adulthood shows high levels of comorbidity (Bolea-Alamañac et al., 2014). In childhood comorbidities include tic disorders, depression, anxiety, learning difficulties, ASD, dyslexia, obsessive compulsive disorder, bipolar disorder, conduct and substance use

disorders, obesity, personality disorders and difficulties with motor coordination (developmental coordination disorder (DCD)) (Bolea-Alamañac et al., 2014; Germanò, Gagliano, & Curatolo, 2010; Taurines et al., 2010). In an analysis of the 2007 National Survey of Children's Health on 61,779 children including 5028 with ADHD, 33% of those with ADHD had one comorbid disorder, 16% had 2 and 18% had 3 or more. Parent report showed a significantly higher level of comorbid problems compared to the non-ADHD children, for example, 46% vs 5% had a learning disability and 18% vs 2% had anxiety (Larson, Russ, Kahn, & Halfon, 2011). Comorbidity was associated with a range of impairment including problems with school, socialising and family relations. Children's functioning declined with increasing numbers of comorbid conditions, and use of health and educational services increased (Larson et al., 2011).

Several studies have suggested that those whose ADHD persists into adulthood have more severe symptoms and are more impaired than those who remit. For example, higher ADHD symptom severity and the presence of comorbid disorders (particularly mood, anxiety and conduct disorders) are commonly found to predict persistence (Biederman et al., 1996, 2011; Kessler, Adler, Barkley, et al., 2005; Lara et al., 2009; Molina et al., 2009). Around 75% of adult cases are said to have at least one comorbid condition (Kooij et al., 2010). Comorbidities in adulthood include substance abuse, ASD, dyslexia, learning difficulties, personality disorder, depression, anxiety and bipolar disorder (Bolea-Alamañac et al., 2014; Kessler et al., 2006). In a general population sample of 3,199 adults the estimated rate of ADHD was 4.4%. Those with ADHD had significantly higher rates of comorbid disorders compared to those without ADHD (mood disorders: 38.3% ADHD vs 11.1% non-ADHD, anxiety disorders: 47.1% vs 19.5%, substance use disorder: 15.2% vs 5.6%, intermittent explosive disorder: 19.6% vs 6.1%) (Kessler et al., 2006). Rates of ASD are also high: one multicentre study in adults found that 43% of adult patients diagnosed with autism had ADHD symptoms (Hofvander et al., 2009).

Substance abuse is highly prevalent in ADHD (Bolea-Alamañac et al., 2014; Kessler et al., 2006). For example in 1761 adults with substance dependence, 5.22% had a diagnosis of ADHD versus 0.85% of individuals without substance dependence (Arias et al., 2008). In a smaller study (n=120) of adults with ADHD, lifetime risk for substance use disorder (SUD) was as high as 52% versus 27% in controls (Biederman et al., 1995). In a 10 year follow-up study of children with and without ADHD,

ADHD was found to be a significant risk factor for the development of substance use disorder (Wilens et al., 2011). Research in non-clinical samples have found higher rates of substance abuse (compared to those without ADHD symptoms) in those with undiagnosed ADHD or with high levels of ADHD symptoms (Gudjonsson, Sigurdsson, Sigfusdottir, & Young, 2012; Young & Thome, 2011). In 10,987 adolescents, those with high levels of ADHD symptoms reported significantly higher rates of substance abuse compared to their non-ADHD peers (Gudjonsson et al., 2012). This suggests that those with undiagnosed ADHD may be attempting to self-medicate (Bolea-Alamañac et al., 2014). This will be discussed further in Section 1.3.2.

1.1.6 Impairment

ADHD in both childhood and adulthood is a severe and impairing disorder. It is associated with increased rates of unemployment (Halmøy, Fasmer, Gillberg, & Haavik, 2009), sickness absence (de Graaf et al., 2008), drug and alcohol abuse (Kaye, Darke, & Torok, 2013), lack of academic achievement and higher rates of poor social adjustment, family or marital conflict (Biederman et al., 2006; Fried et al., 2013; Wymbs, Jr, Gnagy, & Wilson, 2009). The excess cost of the condition (in terms of education, occupational impairment and medical treatment) was estimated at over $30 billion in the United States in 2000 (Birnbaum et al., 2005).

More recent research has focused on the link between ADHD and criminality. Meta-analysis estimated a 25.5% prevalence of ADHD in prison populations (Young, Moss, Sedgwick, Fridman, & Hodgkins, 2014). Compared with the prevalence in the general population, this translated to a five-fold increase in ADHD in youth prison populations (30.1%) and a 10-fold increase in adult prison populations (Young et al., 2014). Increased criminality in ADHD could be mediated by comorbid personality disorders, particularly antisocial personality disorders, as high rates of personality disorder are reported in prison populations (Rösler et al., 2004). Up to one third of personality disordered offenders screen positive for ADHD (Bolea-Alamañac et al., 2014; Young et al., 2011). Treatment of ADHD with stimulants or atomoxetine has, however, been found to reduce offending, suggesting that core ADHD symptoms play a key role in maintaining criminal behaviour. An epidemiological study of data from the Swedish National Register showed an approximately six-fold increase in criminal convictions associated with ADHD, which were reduced during periods of

targeted treatment for ADHD by around 32% in men and 41% in women; an effect that was not seen for treatment with antidepressants (Lichtenstein et al., 2012).

1.1.7 Heritability

Results from 20 twin studies in children and adolescents (with only one study including young adults) have shown that ADHD is one of the most heritable of psychiatric disorders (~ 76%) indicating a strong genetic architecture (Faraone et al., 2005). Some twin studies, using self-rated ADHD scales, have shown substantially lower heritabilitites in adults (~30–40%) (Boomsma et al., 2010; Larsson et al., 2012; Van Den Berg, Willemsen, De Geus, & Boomsma, 2006). However this may be due to rater-effects resulting from the use of self-report measures in adults, whilst heritability estimates in children are generally based on self and informant reports (Larsson, Chang, D'Onofrio, & Lichtenstein, 2014). More recent twin studies, one of which included adults with a clinical diagnosis, have combined self, parent or clinician ADHD ratings and found equivalent heritability estimates to those found in childhood (~72%-78%) (Chang, Lichtenstein, Asherson, & Larsson, 2013; Larsson et al., 2014). Given the high heritability of ADHD it is associated with high levels of familiality (Faraone et al., 2000; Manshadi, Lippmann, O'Daniel, & Blackman, 1983). First degree relatives of those with ADHD are 2-8 times more likely than relatives of unaffected individuals to also have ADHD (Faraone et al., 2005), and may be around 10-fold when probands are restricted to the more severe combined type diagnosis (Chen et al., 2008). Adoption studies have also found biological relatives of hyperactive children to show more ADHD traits than adoptive relatives (e.g. Cantwell, 1975; for a review see Thapar, Cooper, Eyre, & Langley, 2013).

1.1.8 Categorical versus dimensional approaches to diagnosis

The categorical classification of ADHD and other mental disorders in the DSM 5 has been criticised as failing to account for the heterogeneity of mental illness. It has therefore been argued that the classification of ADHD and other disorders may be better represented dimensionally. Dimensional classification describes the classification of a disorder along a continuum of symptoms rather than discrete categories (Brown & Barlow, 2005). In line with this, evidence from quantitative genetic studies have suggested that ADHD may represent the extreme of one or more continuously distributed trait (Chen et al., 2008; Levy, Hay, McStephen, Wood, & Waldman, 1997). For example, estimates of heritability remained high (0.75-0.91) regardless of whether ADHD was defined as a

continuum or discrete disorder (Levy et al., 1997). A recent review highlighted guidelines from the US National Institute of Mental Health which encourages researchers to use a dimensional approach when examining the cognitive and clinical features of ADHD and other disorders (Faraone et al., 2015; Sanislow et al., 2010). Therefore, as a whole, both categorical and quantitative approaches are encouraged in the investigation of ADHD.

1.1.9 Aetiology of ADHD

1.1.9.1 Genetic studies

Given the high heritability of ADHD, research has focused on elucidating the genetic architecture of this complex polygenic disorder (Gizer, Ficks, & Waldman, 2009). Early candidate gene studies (which test for association between ADHD and genes thought likely to be involved in its pathophysiology) focused on neurotransmitter systems, identifying a number of genes involved in dopaminergic and serotonergic neurotransmission (Faraone et al., 2005; Gizer et al., 2009; Li, Sham, Owen, & He, 2006; Smith, 2010; Yang et al., 2007). The strongest evidence of association from these studies is with the 7-repeat allele of the dopamine D4 receptor gene (Li et al., 2006). More recently converging evidence has further suggested that genetic variation within a network of genes involved in neural growth increases risk for ADHD (Poelmans, Pauls, Buitelaar, & Franke, 2011).

Genome wide association studies (GWAS) explore the whole genome for common genetic variants. It was recently estimated that 28% of the variance in ADHD is explained by currently available genome-wide genetic marker arrays (Yang et al., 2013). In polygenic risk score analysis, the genetic signals attributed to common variants derived from a discovery sample are used to predict phenotypic effects in a second sample (Faraone et al., 2015). The polygenic risk for clinically diagnosed ADHD broadly predicts ADHD symptoms in the population (Martin, Hamshere, Stergiakouli, O'Donovan, & Thapar, 2014). This suggests that the genes regulating the diagnosis of ADHD also regulate the expression of subclinical levels of ADHD symptoms (Faraone et al., 2015). This supports conclusions from family and twin studies which suggest that ADHD is an extreme and impairing tail of one or more heritable quantitative traits (Chen et al., 2008; Henrik Larsson, Anckarsater, Råstam, Chang, & Lichtenstein, 2012). Genetic overlap with other disorders has also

been found. For example, a combined GWAS of ADHD, autism spectrum disorders, depression, bipolar disorder and schizophrenia identified four shared genome-wide significant loci (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013).

Increased large, rare chromosomal deletions and duplications known as copy number variants (CNVs) have also been found in ADHD (Elia et al., 2012; Stergiakouli et al., 2012; Williams et al., 2010, 2012). The first study to report case control differences for CNVs in ADHD found 15.6% of patients with ADHD carry large CNVs of > 500,000 base pairs in length, compared to 7.5% of individuals without the disorder (Williams et al., 2010), although similar presentations have been found in children with ADHD with and without such CNVs (Langley et al., 2011), and there is overlap of CNVs spanning the same genes in other psychiatric (neurodevelopmental) disorders (e.g. autism, schizophrenia) (Williams et al., 2010). Therefore increased CNV burden is not unique to ADHD but may represent vulnerability to several psychiatric, particularly neurodevelopmental, disorders.

1.1.9.2 Environmental associations

Although a number of environmental factors have been associated with ADHD it is difficult to distinguish correlation from causality (see Thapar et al., 2013 for a review). Prematurity and severe early deprivation appear to be the most conclusive risk factors to date with only severe early deprivation likely to be on the causal pathway (Bolea-Alamañac et al., 2014; Thapar et al., 2013). There is suggestive but not conclusive evidence for an association with maternal smoking/alcohol/substance misuse and stress during pregnancy, low birth weight, family adversity and low income, parent-child hostility and severe early deprivation. However factors such as maternal smoking during pregnancy may reflect gene-environment correlation, since there is evidence that the association with offspring ADHD does not remain once genetic or other potential confounders are controlled for (Langley, Heron, Smith, & Thapar, 2012; Thapar et al., 2009). A number of dietary factors (nutritional deficiencies (e.g. omega-3) or surpluses (e.g. sugar)) and environmental toxins (e.g. lead, NO_2) have also been linked (Bolea-Alamañac et al., 2014; Thapar et al., 2013). Research into environmental factors is an important future direction.

1.1.9.3 Gene x environment interaction

Heritability estimates also include the interplay between the gene x environment (Rutter, 2006). Genetic risk may influence susceptibility to ADHD by altering individual sensitivity to environmental risks or protective factors. Preliminary research has found that the dopamine and serotonin systems may interact with psychosocial risk factors (e.g. marital conflict/adversity/stress) and the dopamine system with pre-natal risk factors (e.g. maternal smoking) (see Nigg, Nikolas, & Burt, 2010 for review). For example, a variant of the 5-HTTLPR, a polymorphic region located in the promoter of SLC6A4, is involved in the hyperactivity/impulsivity dimension of ADHD in interaction with stress (van der Meer et al., 2014). However replication of these results is necessary before any conclusions can be drawn (Thapar et al., 2013). More recent work has focused on the effect of the environment on gene expression, known as epigenetics (Mill & Petronis, 2008). For example, the proposed protective effect of exercise in ADHD could be mediated by alterations in gene expression (Rommel, Halperin, Mill, & Asherson, 2013; Rommel et al., 2015). Future research in this area may provide new insights into the pathogenesis of ADHD (Mill & Petronis, 2008).

1.1.9.4 Neuroimaging studies

The exact neurobiological mechanisms underlying ADHD are poorly understood. One recent theory has focused on the association between ADHD and abnormal brain connectivity, for example within the brain's default mode network which has been linked to attention regulation (Castellanos & Proal, 2012; Castellanos et al., 2008; Konrad & Eickhoff, 2010). Several neural networks have been suggested to play a key role, although as yet there is very little in the way of causal modelling to convincingly demonstrate which reflect the key neural deficits leading to the symptoms and impairments of ADHD. These neural pathways include: (1) reduced connectivity in the ventral fronto-striato-parietal (cognitive control) circuit; (2) reduced connectivity in the dorsal fronto-striato-parietal attention circuit; (3) reduced connectivity in inferior fronto-supplementary–motor-area-parieto-cerebellar networks for timing function; (4) reduced connectivity in orbitofrontal-ventral striatal (salience/reward) circuit; (5) reduced connectivity between the default mode network (DMN) and cognitive control circuits, reduced deactivation of the DMN during cognitive tasks and reduced connectivity between components of the DMN (Cortese et al., 2012; Faraone et al., 2015).

Evidence from electroencephalography (EEG) studies has indicated increased theta activity and an increased theta to beta ratio in children and adults with ADHD compared to controls. This is said to indicate cortical underactiviation in ADHD due to the association between theta activity and drowsiness (Loo & Makeig, 2012; Tye, McLoughlin, Kuntsi, & Asherson, 2011), and has been linked to cognitive theories of sub-optimal arousal in ADHD (see Section 1.1.10.1). This finding has recently led to the approval of an EEG-based diagnostic aid for ADHD in children which calculates the theta to beta ratio (FDA, 2014). However this has been criticised given that an increased theta/beta ratio is not a robust finding; it is estimated that up to 16% of children with ADHD will have typical theta/beta ratios (Bolea-Alamañac et al., 2014; Loo & Makeig, 2012)

Previously, longitudinal data suggested delayed cortical maturation in children with ADHD (Shaw & Rabin, 2009). Structural and functional brain abnormalities have been found to overlap between children and adults with ADHD (Schneider, Retz, Coogan, Thome, & Rösler, 2006), suggesting that when the disorder persists so do the underlying brain abnormalities. Differences in grey and white matter have been reported (Shaw & Rabin, 2009), with meta-analyses (in children and adults with ADHD) finding various structural differences including: smaller right hemispheric grey matter volumes of the basal ganglia, smaller grey matter volumes in total and right cerebral volume, cerebellum, corpus callosum, frontal lobes, prefrontal cortex, deep frontal white matter and temporal lobe and potential grey matter increases in the left posterior cingulate cortex/precuneus (Ellison-Wright, Ellison-Wright, & Bullmore, 2008; Frodl & Skokauskas, 2012; Nakao, Radua, Rubia, & Mataix-Cols, 2011). Although results from these meta-analyses are inconsistent, evidence for reductions in grey-matter in the dopamine-rich basal ganglia appears the most robust (Bolea-Alamañac et al., 2014).

Single photon emission computed tomography (SPECT) studies have revealed altered dopamine receptor binding and potential differences in the density of the dopamine transporter in ADHD, particularly in the striatum (del Campo, Müller, & Sahakian, 2012; Hesse, Ballaschke, Barthel, & Sabri, 2009; Krause, la Fougere, Krause, Ackenheil, & Dresel, 2005; Krause, Dresel, Krause, Kung, & Tatsch, 2000). These findings support the well-established dopamine theory of ADHD, suggesting dysfunction in the dopamine neurotransmitter system may interfere with neuropsychological

functions such as in the domains of attention and motivation (Swanson et al., 2007). The main evidence for this is based on the effectiveness of treatment with stimulant medication (i.e. methylphenidate; see also Section 1.1.11 for discussion of treatment) that block the brain dopamine transporters, significantly enhancing extracellular dopamine (Volkow, Fowler, Wang, Ding, & Gatley, 2002).

1.1.10 Cognitive deficits in ADHD

A heterogeneous range of neurocognitive impairments are present in both children and adults with ADHD and are summarised in Table 1-1. Impairments include deficits in executive function (Sonuga-Barke, Sergeant, Nigg, & Willcutt, 2008), reaction time variability (RTV) (Kofler et al., 2013), difficulties in state regulation (indexed by greater sensitivity to rewards (discussed below (Section 1.1.10.1.)) (Sonuga-Barke & Fairchild, 2012), and impaired temporal information processing (such as a reduced ability to discriminate between two time intervals that differ in duration (such as 500ms vs 600ms)) (Toplak, Dockstader, & Tannock, 2006; Toplak & Tannock, 2005).

The most consistent deficits in ADHD are thought to be in executive function and RTV (Bolea-Alamañac et al., 2014; Kofler et al., 2013). Executive function, mainly associated with the prefrontal cortex, refers to the completion of goal directed tasks through the combination of higher order cognitive processes (Sonuga-Barke et al., 2008). In children and adults with ADHD, meta-analyses have found deficits in executive function to include measures of attention, response inhibition, vigilance working memory and planning (Frazier, Demaree, & Youngstrom, 2004; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). For example, omission errors (where a participant fails to respond when a response is required) and commission errors (where a participant responds on a task where a response is not required) are thought to measure sustained attention and response inhibition respectively (Frazier et al., 2004). Numerous studies have reported increased commission and omission errors on computerised attention tasks in both children (Willcutt et al., 2005) and adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD (for reviews see Bolea-Alamañac et al., 2014; Frazier et al., 2004). Reaction Time Variability (RTV) is another highly investigated cognitive deficit in ADHD. Thought to be a measure of attentional lapses, impairments are consistently found in children (Andreou et al., 2007; Tye et al., 2013; Uebel et al., 2010) and

adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD (for reviews see Frazier et al., 2004; Karalunas, Geurts, Konrad, Bender, & Nigg, 2014; Kofler et al., 2013).

1.1.10.1 State regulation in ADHD

One theory of the cognitive deficits in ADHD is proposed by the 'cognitive energetic model'. This model proposes deficits in cognition to be the result of a reduced energetic state (Sergeant, 2000). In line with this, several studies in ADHD children have reported a significantly greater improvement in Mean Reaction Time (MRT) and RTV following the introduction of rewards and/or an increase in presentation rate on computerised attention tasks (Andreou et al., 2007; Cheung et al., under review; Slusarek et al., 2001; Uebel et al., 2010). Meta-analysis has estimated a small but significant effect (g=0.3-0.4) of incentives in reducing RTV (Kofler et al., 2013). Evidence for sensitivity to rewards in adults with ADHD is limited, with one study showing no effect of rewards on cognitive measures (Ströhle et al., 2008), and few others conducted; this is an area for future research.

Table 1-1: Summary of the cognitive deficits found in ADHD in children, adolescents and adults (adapted from Bolea-Alamañac et al., (2014))

	Child/adolescent		**Adult**	
	Quantitative reviews	**Strength of effect**	**Quantitative reviews**	**Strength of effect**
Executive function				
CPT commission errors	Yes	Moderate	Yes	Moderate
CPT ommission errors	Yes	Moderate	Yes	Moderate
SST-Reaction Time	Yes	Moderate	Yes	Small
Spatial working memory	Yes	Moderate	Yes	Small
Verbal working memory	Yes	Moderate	Yes	Small
ToL/H	Yes	Moderate	Yes	Small
Trials-B	Yes	Moderate	Yes	Moderate
Stroop Inteference	Yes	Small	Yes	Small
Reaction time				
RTV	Yes	Moderate/Large	Yes	Moderate
Timing				
Time discrimination tasks	No	Moderate	No	Small/moderate
Time reproduction tasks	No	Moderate	No	Small/moderate
State regulation				
Varying event rate	No	Moderate	No	Small
Reward	No	Small	No	No effect
Punishment	No	Small	No	No effect

Note. Quantitative studies column describes the existence of quantitative reviews published for the named test. **Strength of effect**: no effect, Cohen's d < .2, small effect, Cohen's d .2–.4; moderate effect Cohen's d .4–.7; large effect Cohen's d .7–1.0; very large effect Cohen's d > 1.0.

Task domains in table:

Executive Function (Sonuga-Barke et al., 2008): CPT = continuous performance test of sustained attention; SSRT = Stop signal reaction time measure of inhibitory control; Tol/H = Tower of London/Hanoi: measure of planning; Trials-B = a measure of planning; Stroop interference = a measure of attention/processing speed.

Reaction time (Hervey, Epstein, & Curry, 2004; Kofler et al., 2013)**:** RTV = Reaction time variability - Variation in Mean Reaction Time, often measured on CPT tasks.

Timing (temporal information processing) (Toplak et al., 2006): Time discrimination tasks = the participant is required to discriminate between two brief time intervals. Time reproduction tasks = the participant is required to reproduce a specified time interval.

State Regulation (Andreou et al., 2007; Sonuga-Barke, Bitsakou, & Thompson, 2010): ISI effects = Greater sensitivity to the effect of varying event rate on performance; Reward = measure of sensitivity to the effects of adding rewards to tasks; Punishment = measure of sensitivity to the effects of adding punishments to tasks.

1.1.10.2 Cognitive deficits and diagnosis

Although the DSM-5 includes cognitive deficits as an associated feature of ADHD (American Psychiatric Association, 2013) these deficits are heterogeneous, present in some but not all cases, and seen in controls and other mental health conditions (such as ASD) (Rommelse, Geurts, Franke, Buitelaar, & Hartman, 2011). They therefore lack the sensitivity and specificity to be used as accurate diagnostic tools (Bolea-Alamañac et al., 2014; Doyle et al., 2005). For example, tests for executive function lead to a high level of false negative cases (Nutt et al., 2007). The association between cognitive impairments and ADHD is sometimes taken to imply a causal relationship. In support of this, research has found a familial overlap between ADHD symptoms and RTV, indicating potential shared genetic underpinnings (Kuntsi et al., 2010). However it is also proposed that many of the cognitive impairments associated with ADHD may arise as a consequence of the many potential outcomes of aetiological influences on ADHD (for example genetic pleiotropy), or may reflect epiphenomena of the disorder (Kendler & Neale, 2010).

Given the heterogeneity of cognitive impairments in association with ADHD symptoms, research has suggested that multiple different mechanisms might underlie the symptoms and impairments of ADHD (Coghill, Rhodes, & Matthews, 2007). Greater treatment effects (with stimulant medication) have been found for symptoms than cognition (Banaschewski et al., 2006; Coghill et al., 2014; Faraone & Buitelaar, 2010). Studies have also found a dissociation of the treatment effects on ADHD symptoms and cognitive performance, such that change in response to treatment does not appear to be correlated between cognitive and ADHD symptom measures (Bédard et al., 2014; Coghill et al., 2007; K. P. Schulz, Fan, & Be, 2014). Therefore there is currently little support for a direct causal relationship between symptoms and measures of cognitive performance in ADHD (Bolea-Alamañac et al., 2014). This is discussed in greater detail in the discussion section of Chapter 2 (Cooper, Tye, Kuntsi, Vassos, & Asherson, 2015).

1.1.11 Treatments in ADHD

Stimulant medication is the first line treatment for both children and adults with ADHD, with around 70% of adults showing a positive response and even better responses seen in children (Biederman, Spencer, & Wilens, 2004; Bolea-Alamañac et al., 2014; Medori et al., 2008; Spencer et al., 2005). Psychological treatments (e.g. cognitive behavioural therapy) are also recommended as an adjunctive or (particularly in children) mono-therapy (Young & Amarasinghe, 2010), with evidence suggesting the combination of pharmacological and psychological treatment to be superior to medication alone (Solanto et al., 2013; Young & Amarasinghe, 2010). For pharmacological treatment, meta-analyses have found medium to high (d = ~0.6 – 0.9) effect sizes for stimulant and non-stimulant medication (Faraone & Buitelaar, 2010; Faraone & Glatt, 2010; Mészáros et al., 2009) with effect sizes above 1 for higher doses (Faraone, Spencer, Aleardi, Pagano, & Biederman, 2004). In the most recent meta-analyses of drug treatments in adult ADHD, effect sizes were reported in to be in the region of d = 0.4 – 0.8 (Castells, Ja, Bosch, Nogueira, & Casas, 2011; Castells et al., 2011; Cunill, Castells, Tobias, & Capella, 2013; Koesters, Becker, Kilian, Fegert, & Weinmann, 2009). Pharmacological treatment has been found to improve quality of life and daily functioning in addition to ADHD symptoms (Surman, Hammerness, Pion, & Faraone, 2013).

The main mechanism of action of ADHD drugs is said to be through enhancement of dopaminergic and noradrenergic neurotransmission in brain areas associated with attention and impulsivity, including the prefrontal cortex, the striatum and the cerebellar vermis (Anderson, Polcari, Lowen, Renshaw, & Teicher, 2002; Arnsten & Dudley, 2005; Bolea-Alamañac et al., 2014; Volkow, Fowler, Wang, Ding, & Gatley, 2002). Meta-analysis of imaging studies has found stimulant medication to be associated with normalisation of structural abnormalities in ADHD (Nakao et al., 2011). In our own work, we found normalisation of very low frequency electroencephalography (VLF-EEG), thought to reflect synchronisation of neural oscillations within the brains organisational networks (Helps, Broyd, James, Karl, & Sonuga-Barke, 2009), after treatment with methylphenidate in adults with ADHD (Cooper et al., 2014).

1.1.11.1 Difficulty in obtaining treatment

Despite clear guidelines from NICE (NICE, 2008), there is difficulty in obtaining a diagnosis and treatment for adults with ADHD (Bolea-Alamañac et al., 2014). This was a significant observation in the samples recruited for this thesis and is discussed in Chapter 7, Section 7.6.1. In the UK less than 10% of adults with ADHD who require medication are thought to receive treatment (Bolea-Alamañac et al., 2014). Primary care prescription records show the use of medication to rapidly reduce once adolescents reach 16-18 years, with a significant discrepancy between numbers meeting full criteria for the disorder and numbers prescribed medication (McCarthy et al., 2009; McCarthy et al., 2012). A lack of treatment for ADHD is associated with significant impairment. One marked example is the high rate of ADHD in prison populations which is significantly higher than in the general population (estimated at 25.5%) (see Section 1.1.6), yet we know that very few prisoners with ADHD are being treated for the condition (Young et al., 2014; Young & Thome, 2011), despite evidence that this is likely to reduce impairment (Lichtenstein et al., 2012).

A number of factors may contribute to the difficulty in obtaining a diagnosis (Kooij et al., 2010):

1) There may be unease within some mental health professionals in the identification and treatment of ADHD (Bolea et al., 2012; Singh, 2008). For example, some hold the erroneous view that ADHD is a construct manufactured by the pharmaceutical industry (Goldstein, 2006).
2) Lack of training in ADHD.
3) Clinicians may be reluctant to widen the already overstretched psychiatric services to include adults with ADHD (Bolea et al., 2012).

Difficulties in obtaining the adult diagnosis and recommendations for improvement have been highlighted numerous times (Bolea et al., 2012; Bolea-Alamañac et al., 2014; Kooij et al., 2010; Nutt et al., 2007), yet with the significant issues found in the samples recruited for this thesis, it is clear that this remains a pertinent issue.

1.1.11.2 Why is there a need to investigate alternative treatments?

Pharmacological medication is the first line treatment for ADHD. There are however a number of potential problems with the currently available medications, including non or partial response and

adverse effects. Not all patients are tolerant of the main drugs used to treat ADHD. Common side effects of stimulants include sleeplessness, loss of appetite, weight loss, lethargy, dysphoria and irritability (Faraone et al., 2015; Leonard, McCartan, White, & King, 2004; Sangal et al., 2006). A set of overlapping problems are also seen with atomoxetine which may have additional problems, such as erectile dysfunction. Serious adverse events may include the onset of tics, acute anxiety states, depression, psychosis and mania (Faraone et al., 2015). In children, stimulants are also associated with small delays in growth (Faraone, Biederman, Morley, & Spencer, 2008). The recommended treatments for ADHD can all increase blood pressure, and this can present as a problem in some cases. These more serious adverse-effects of stimulant treatment led to US Food and Drug Administration (FDA) warnings. From February 2007 all FDA-approved drug treatments for ADHD (methylphenidate, dexamphetamine and atomoxetine) have carried warnings that their use could involve risks of cardiovascular effects, growth suppression and the development of psychosis or other psychiatric conditions. Rare cases of sudden death have also been reported amongst children using stimulant medications for ADHD. The FDA warns that the use of these medications by children with heart conditions should be avoided or undertaken with great caution (FDA, 2006).

Participants recruited for this thesis reported a number of problems with stimulant medication. One said that the medication made him become aggressive to the point that his marriage broke down: *"Completely unable to control my temper".*

A number of participants felt that they lost their personality when on the medication or had a sense of depersonalisation: *"It felt as if between me and the world was a plastic window. I was happy and content, but didn't feel the urge anymore to show that. Friends/family told me that I had lost my spark."*

Some felt that they did not want to take pharmacological medication in order to function: *"Damaged my self-esteem…can I really only function if I am on drugs?"*

Some did not respond to the medication and research has found around 25% of patients are treatment resistant (Biederman et al., 1999): "*I was also disappointed a bit as I had hoped I could suddenly concentrate a lot more, to me it didn't feel like it did improve."*

There are, in addition, questions over the long-term efficacy and the potential for abuse of stimulants (Bolea-Alamañac et al., 2014; Dunnick & Hailey, 1995; Nasrallah et al., 1986). There are also concerns over effects on physical health and (particularly in children) developmental effects: *"I was (and am) concerned the effect it would have on for instance my kidneys over longer periods of time (and possibly pregnancy)."*

Given these problems with adverse events, partial response and long-term efficacy, it is important that alternative treatments for ADHD are investigated.

1.1.12 Interim summary

ADHD is a persistent neurodevelopmental disorder characterised by deficits in inattention, hyperactivity/impulsivity, cognition and EL. ADHD affects around 5% of children and 2.5% of adults. It is associated with increased risk of comorbid psychiatric disorders and psychosocial impairments, including substance use disorder, educational, occupational and social problems and criminality. ADHD is highly heritable suggesting a strong genetic component. Although associations are starting to be identified with common genetic variants and rare CNVs, there is no single risk factor for ADHD. Genetic and environmental factors appear to act together to increase susceptibility. Neuroimaging studies have found numerous structural and brain abnormalities and more recent research has focused on abnormal brain connectivity in ADHD, yet it remains unclear which might play a causal role. Given the marked response to dopamine enhancing stimulant medications, abnormality of the dopamine system is a well-established hypothesis of ADHD. However, there are a number of problems with available medications, including both stimulants and atomoxetine, which includes both serious and common adverse events and partial or non-response. Research into alternative treatments therefore remains vital.

1.2 Alternative treatments in ADHD: Omega-3 polyunsaturated fatty acid supplementation

Omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation is an extensively studied alternative treatment for ADHD (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). Thought to affect monoamine neurotransmission (Chalon, 2006), numerous case/control and intervention studies have provided evidence for a small but significant benefit of *n*-3 PUFA in children with ADHD (Hawkey & Nigg, 2014; Bloch & Qawasmi, 2011; Sonuga-Barke et al., 2013). Given that *n*-3 PUFA supplementation has a significantly lower adverse effect profile than pharmacological treatment it has been concluded that it may be useful as an adjunctive treatment or monotherapy for those who do not wish to take pharmacological treatment.

1.2.1 Physiological effects on *n*-3 PUFA

Long-chain polyunsaturated Fatty Acids (PUFAs) are thought to be the most important for health and development, making up at least 35% of the 50-60% lipid content of the human brain (Gow & Hibbeln, 2014). The two major *n*-3 PUFAs are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Gow & Hibbeln, 2014). PUFAs cannot be synthesised by humans and must be either ingested or metabolised from their fatty acid precursors (Elmadfa & Kornsteiner, 2009; Haag, 2003; Innis, 2008). EPA and DHA are metabolised from their parent compound alpha linoleic acid (ALA). Whereas Omega-6 PUFAs (*n*-6 PUFA) are metabolised from their parent compound linoleic acid (LA) (Brenna, Salem, Sinclair, & Cunnane, 2009). This metabolism process is shown in Figure 1-1.

EPA and DHA are thought to exert their effects through a number of important functions related to neural activity and brain development. This includes aiding cell membrane fluidity, neurotransmission, ion channels, enzyme regulation, gene expression, myelination, neurogenesis and synaptogenesis (Gow & Hibbeln, 2014; Gow, Hibbeln, & Parletta, 2015; Innis, 2008; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001; Janssen & Kiliaan, 2014). For example, DHA is the most abundant PUFA in the brain, making up 30% of the lipid cell membranes in grey matter, and may be important for optimal neural functioning (Brenna & Diau, 2007; Diau et al., 2005; Gow & Hibbeln, 2014). PUFAs are also important building blocks of neuronal membranes; DHA and EPA being the main components of these membranes (Janssen & Kiliaan, 2014).

PUFAs have also been linked to inflammation through production of eicosanoids. Eicosanoids are lipid mediators that are involved in a wide array of physiological functions, including inflammation (Janssen & Kiliaan, 2014; Schmitz & Ecker, 2008; Simopoulos, 2011). It is thought that, through production of different eicosanoids, *n*-3 PUFAs may limit neuroinflammation whereas *n*-6 PUFAs may promote inflammation (Gow & Hibbeln, 2014; Sinn, Milte, & Howe, 2010; Janssen & Kiliaan, 2014; Simopoulos, 2002). Inflammation has been linked to a number of mental health problems including ADHD, depression, schizophrenia and bipolar disorder (Dean, 2011; Raison & Miller, 2013; Strickland, 2014).

A balanced ratio of *n*-6 to *n*-3 PUFA is therefore required for optimal health and development (Hawkey & Nigg, 2014). However, a current deficit in *n*-3 intake in the western diet is well established (Gow & Hibbeln, 2014; Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010; Simopoulos, 1991). Increasing consumption of vegetable oils and processed foods and decreasing consumption of fish, nuts and seeds has decreased the ratio of *n*-6:*n*-3 from around 1.1:2.1 to approximately 20:1 (Kuipers et al., 2010; Simopoulos, 2002). Furthermore, excessive *n*-6 intake may inhibit *n*-3 PUFA synthesis, reducing the availability of EPA and DHA (Gow & Hibbeln, 2014; Nakamura & Nara, 2004).

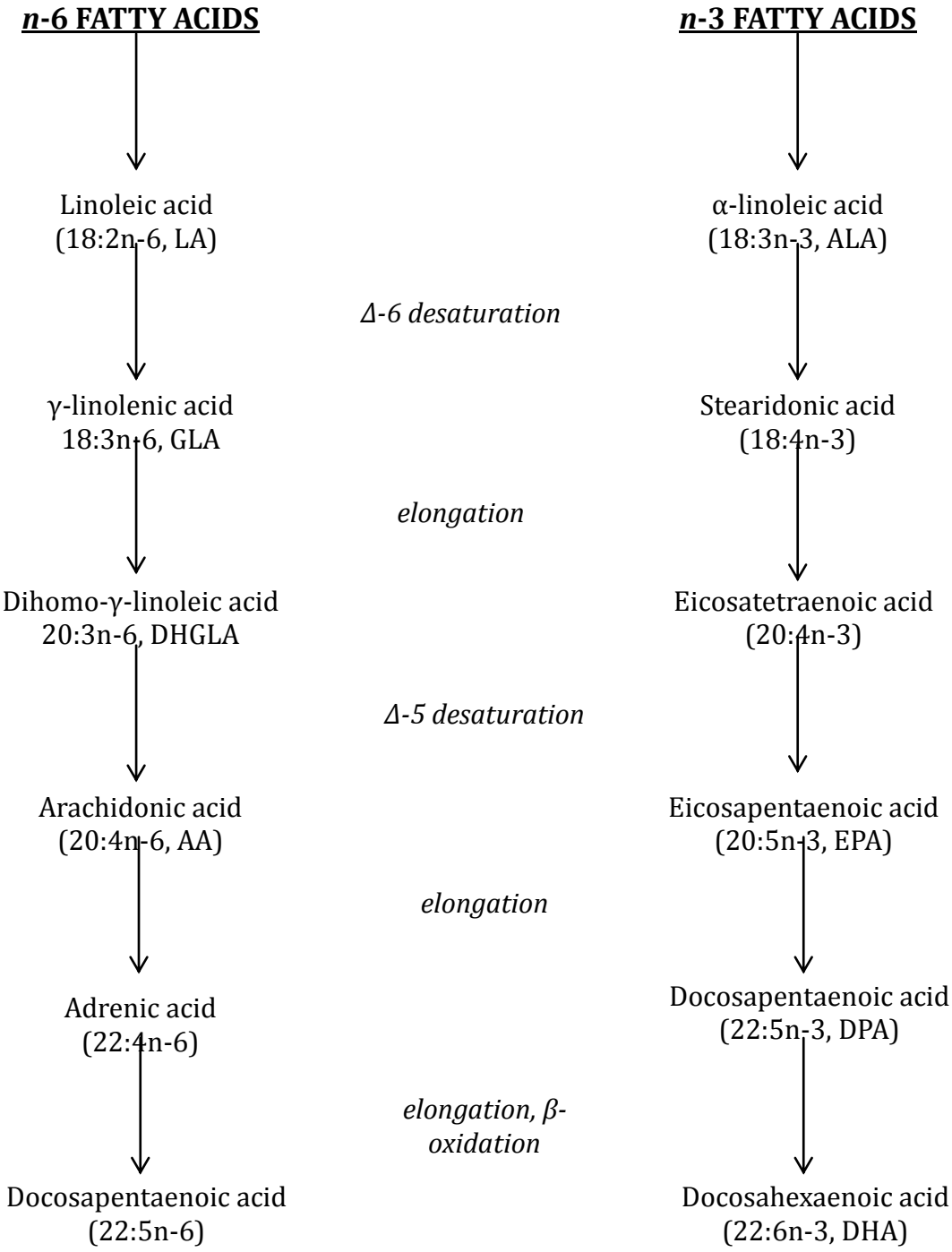

Figure 1-1: Conversion of polyunsaturated fatty acids to their longer-chain metabolites (reproduced from Decsi & Kennedy, (2011))

1.2.2 PUFA in the pathophysiology of ADHD

It is relatively well established that blood levels of *n*-3 PUFA have been found to be lower in ADHD cases compared to controls (Hawkey & Nigg, 2014). A recent meta-analysis in 9 studies (n=586) compared blood (plasma and/or red blood cells) omega-3 levels in children and adults with ADHD to controls (Hawkey & Nigg, 2014). Results showed ADHD cases to have significantly lower total *n*-3 PUFA (EPA, DHA, ALA and DPA) blood levels compared to controls (g = 0.42, $p < .001$). The largest differences were found when looking at DHA alone (g = 0.59, p < .001) and EPA and DHA alone (g= 0.51, $p < 0.001$). Absence of heterogeneity and publication bias increased the reliability of these findings. Alongside this a number of studies have found a higher *n*-6:*n*-3 ratio in children and adults with ADHD compared to controls (Chen et al., 2004; Stevens et al., 1995; Germano et al., 2007; Antalis et al., 2006). It is unclear as to whether differences in *n*-3 PUFA may be due to low dietary intake or an abnormality in PUFA metabolism (Gow & Hibbeln, 2014). Hawkey & Nigg (2014) found some evidence of reduced *n*-3 PUFA intake although firm conclusions could not be drawn (Hawkey & Nigg, 2014). It has been suggested that a genetic vulnerability could lead to disruption in PUFA metabolism (Brookes et al., 2006; Hawkey & Nigg, 2014). However further research is required before any conclusions can be drawn.

1.2.3 Effects of *n*-3 PUFA deficiency

Through alterations of cellular communication, deficiencies in *n*-3 PUFA have been linked to altered neurotransmission particularly for dopamine and serotonin (Assisi et al., 2006; Chalon, 2006; Haag, 2003; Young & Conquer, 2005). Omega-3 deficient animals have shown altered dopamine and serotonin neurotransmission in the frontal cortex and nucleus accumbens, brain areas implicated in ADHD (Delion, Chalon, Guilloteau, Besnard, & Durand, 1996; Bolea-Alamañac et al., 2014; Zimmer et al., 1998; Zimmer et al., 2000; Zimmer et al., 2002). For example, one study found a 40-60% decrease in dopamine in the frontal cortex of *n*-3 deficient rats (Delion et al., 1994).

As discussed in Section 1.1.9.4, the theory that ADHD may be characterised by atypical dopamine levels is well established, supported mainly by the clinical response to dopamine-altering ADHD stimulant medication (Bolea-Alamañac et al., 2014), as well as imaging (e.g. del Campo et al., 2012), and genetic (Li et al., 2006) studies. Serotonergic genes have also been implicated in ADHD (Faraone et al., 2005; Gizer et al., 2009). Alterations in neurotransmission may also contribute to

altered cortical organisation and connectivity, an emerging theory of ADHD (Castellanos & Proal, 2012; Grayson, Kroenke, Neuringer, & Fair, 2014; Hibbeln, Ferguson, & Blasbalg, 2006). Animal studies have found pre and postnatal DHA insufficiency to be associated with a variety of structural changes, such as delayed neuronal migration, disrupted dendritic arborisation and abnormal neuronal development in the hippocampus (Yavin, Himovichi, & Eilam, 2009).

1.2.4 PUFA and other psychiatric disorders

Omega-3 PUFA has also been investigated as a treatment for depression, schizophrenia and autism spectrum disorder (ASD). A number of meta-analyses have confirmed that there seems to be a small to medium effect of *n*-3 PUFA supplementation in improving symptoms of depression in clinical samples (Appleton, Rogers, & Ness, 2010; Grosso et al., 2014; Martins, Bentsen, & Puri, 2012). Although one meta-analysis found no benefit of *n*-3 PUFA in treating depression (Bloch & Hannestad, 2011), this has been attributed to methodological issues such as the combining of clinical and subclinical depressive populations (Martins et al., 2012). Meta-analyses have generally found little evidence of an effect of *n*-3 PUFA supplementation in psychosis (Freeman et al., 2006; Fusar-Poli & Berger, 2012; Irving, Mumby-Croft, & Joy, 2006). Randomised controlled trials have found a beneficial effect of *n*-3 PUFA supplementation in children with ASD (Amminger et al., 2007; Yui, Koshiba, Nakamura, & Kobayashi, 2012). Although these were small studies (each included only 13 children) and a meta-analysis is yet to be conducted, therefore the evidence remains inconclusive. The most promising evidence is therefore for the beneficial effect of *n*-3 PUFA in depression, with effects on psychosis appearing to be negligible, and further evidence required for ASD. This is particularly relevant for ADHD which is highly comorbid with both depression and ASD (Bolea-Alamañac et al., 2014; Kessler et al., 2006).

1.2.5 Intervention studies

1.2.5.1 Omega-3 PUFA and ADHD symptoms

It is now relatively well established that *n*-3 PUFA supplementation may have a small to moderate effect on reducing ADHD symptoms in children. To date four meta-analyses, using data from randomised placebo controlled trials, have been conducted. The first found a small but significant effect of *n*-3 PUFA supplementation on reducing ADHD symptoms in 699 (SMD = 0.31, $p < 0.0001$) (Bloch & Qawasmi, 2011) children with ADHD and related disorders (such as developmental

coordination disorder). The second, a Cochrane Review, pooled results separately for different study designs leading to small sample sizes. Their primary finding was of no significant effect of *n*-3 PUFA on ADHD, although a sub-analyses in two studies did show a significant effect (risk ratio = 2.19, 95% CI 1.04-4.62) (Gillies, Sinn, Lad, Leach, & Ross, 2012). Negative outcomes in this review may have been due to small sample sizes. The third found a small effect on reducing symptoms in 827 children with ADHD (SMD=0.21, *p*=0.007) (Sonuga-Barke et al., 2013). The most recent study in 1408 children with ADHD found a small but significant effect in reducing ADHD symptoms ($g = 0.26$, $p < .001$) (Hawkey & Nigg, 2014).

These studies also found EPA to have greater efficacy in reducing ADHD symptoms (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014). This may be because many of the better-designed studies used a supplement containing a high EPA/DHA ratio (Gow et al., 2015). The Bloch paper also pointed out that most of the current trials are underpowered; they found that to have sufficient power ($\beta = 80\%$, two-sided $\alpha=0.05$) to detect a benefit of *n*-3 PUFA an approximate sample size of 330 children would be required. In contrast, the included trials ranged from 26-117 participants. Therefore inconsistent findings are likely due to trials being considerably underpowered, in addition to wide variation in methodology, formulation and dose (Gow et al., 2015).

Therefore *n*-3 PUFA supplementation in children with ADHD appears to improve ADHD symptoms. However whilst this effect appears to be genuine, it is also small in comparison to the effect size of stimulant and non-stimulant treatment in children with ADHD (d=~0.6–0.9) (Faraone & Buitelaar, 2010; Faraone & Glatt, 2010; Mészáros et al., 2009). Therefore *n*-3 PUFA supplementation should either be used as an adjunctive treatment, or for those who are either unwilling or unable to use pharmacological treatment. The effect of *n*-3 PUFA supplementation in adults with ADHD remains unknown as there has yet to be a published treatment trial.

1.2.5.2 Omega-3 PUFA, cognition and emotional lability.

Neurocognitive impairments and EL are characteristic features of ADHD (see Sections 1.1.10 and 1.1.3). Furthermore n-3 PUFA supplements are promoted to the general population as cognitive enhancers. However results from randomised controlled trials (RCTs) examining the effect of *n*-3 PUFA supplementation on cognition (Manor et al., 2012; Milte et al., 2012; Sinn, Bryan, & Wilson,

2008; Stevens et al., 2003) and EL (Manor et al., 2012; Milte et al., 2012; Richardson, Burton, Sewell, Spreckelsen, & Montgomery, 2012; Richardson & Montgomery, 2005; Stevens et al., 2003; Widenhorn-Müller, Schwanda, Scholz, Spitzer, & Bode, 2014) in ADHD have been mixed. These results are discussed in detail in Chapters 2 and 3 which present, to our knowledge, the first systematic review and meta-analysis of the effect of *n*-3 PUFA supplementation on 1) cognition in typically developing populations and those with ADHD and related disorders (Cooper et al., 2015) and 2) EL in children with ADHD (R E Cooper, Tye, Kuntsi, Vassos, & Asherson, 2016).

1.2.5.3 Omega-3 PUFA in adults with ADHD

The physiological effects of *n*-3 PUFAs are thought to be through a number of important functions related to neural activity and brain development (e.g. aiding cell membrane fluidity, neurotransmission, myelination, neurogenesis, synaptogenesis)(Gow & Hibbeln, 2014; Gow, Hibbeln, & Parletta, 2015; Innis, 2008; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001; Janssen & Kiliaan, 2014). The proposed role of *n*-3 PUFA in the developing brain implicates them in the pathophysiology of ADHD in childhood. Given the finding of a small to moderate effect on reducing ADHD symptoms in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), and that ADHD persists into adulthood in around two-thirds of cases (Faraone, Biederman, & Mick, 2006), it is important that the effect of *n*-3 PUFA in adults with ADHD be examined. In adulthood, around 70% of ADHD cases show a positive response to dopamine enhancing stimulant medication (Biederman, Spencer, & Wilens, 2004; Bolea-Alamañac et al., 2014; Medori et al., 2008; Spencer et al., 2005). This suggests that dysfunction in the dopamine neurotransmitter system could be linked to ADHD (Swanson et al., 2007). As discussed in Section 1.2.3, deficiency in *n*-3 PUFA has been linked to altered dopamine neurotransmission (Assisi et al., 2006; Chalon, 2006; Haag, 2003; Young & Conquer, 2005). Furthermore there is evidence to suggest that adults with ADHD have significantly lower levels of *n*-3 PUFA compared to controls (Hawkey & Nigg, 2014). Given this evidence for the potential role of *n*-3 PUFA in adult ADHD, it is important that a treatment trial investigating the effect of *n*-3 PUFA supplementation in adults with ADHD be conducted.

1.2.6 Interim summary

Omega-3 PUFA is an extensively studied alternative treatment for ADHD with benefits also found for depression. The effects of *n*-3 PUFA are thought to be exerted through alterations in monoamine neurotransmission, membrane fluidity and reductions in inflammation. Supplementation is important given that 1) *n*-3 PUFA cannot be synthesised *de novo* and must be provided via the diet; 2) Western diets typically have an increased *n*-6:*n*-3 PUFA ratio which may promote neuroinflammation and 3) Reduced *n*-3 PUFA and an increased *n*-6:*n*-3 PUFA ratio is typically found in children and adults with ADHD. Meta-analyses of placebo-controlled trials have found *n*-3 PUFA supplementation to improve ADHD symptoms in children. Given this, it has been concluded that it may be useful as an adjunctive treatment or monotherapy for those who do not wish to take pharmacological treatment. However the effect of supplementation on related domains of cognition and EL are unclear as are the effects in adults with ADHD. Furthermore, effects on cognition are important to clarify given that supplements are often promoted as cognitive enhancers in the general population, and given that cognitive impairments are characteristic of ADHD.

1.3 Alternative treatments in ADHD: Cannabinoid medication

Cannabis use in ADHD is high with patients reporting beneficial effects on their symptoms and impairments and some reporting a preference for this over their stimulant medication. Given the evidence for lability of the dopamine system in ADHD and that cannabinoids may affect dopamine transmission it is important to investigate the utility of a cannabinoid medication as a treatment for ADHD.

1.3.1 Properties and effects of cannabis

The chemical compounds in cannabis are known as cannabinoids, and it is estimated that cannabis contains 108 different cannabinoids. The two most relevant for psychiatry are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). Δ9-THC can induce acute psychotic symptoms in medicated schizophrenic patients and healthy controls (D'Souza, Sewell, & Ranganathan, 2009; Englund et al., 2012). However CBD is thought to have the opposite effects; it has been found to reduce Δ9-THC induced psychotic symptoms and cognitive impairments (Englund et al., 2012) and has shown promise as a possible antipsychotic (Leweke et al., 2012; Zuardi, Crippa, Hallak, Moreira, & Guimaraes, 2006).

Given this, recent research has found that the established link between cannabis and psychosis (Casadio, Fernandes, Murray, & Di Forti, 2011; Henquet, Murray, Linszen, & Van Os, 2005; Moore et al., 2007) may be specific to cannabis that is high in THC (known as sinsimella (skunk)) but not that which contains similar levels of THC to CBD (known as cannabis resin ('hash')). In a case/control study, 410 patients with first-episode psychosis were compared to 370 controls. A significantly higher risk of developing a psychotic disorder was related to the use of sinsemilla everyday but not to cannabis resin ('hash') (Di Forti et al., 2015). However sinsemilla dominates the UK black market with levels of THC appearing to rise year on year (see
Figure 1-2) and cannabis resin becoming increasingly difficult to obtain (Cascini, Aiello, & Di Tanna, 2012; Potter, Clark, & Brown, 2008).

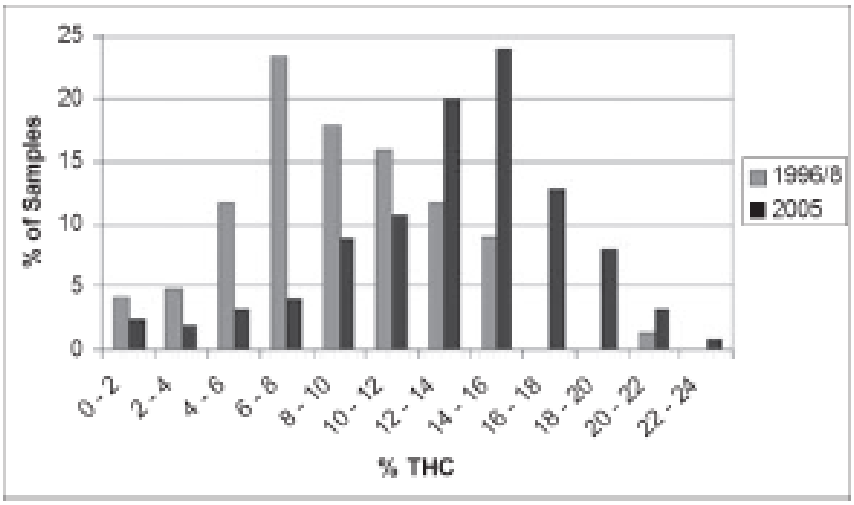

Figure 1-2: A comparison of the ranges of THC contents of Sinsemilla seized in the UK and analyzed by the Forensic Science Service in 1996–8 (n = 145) and samples seized by police in Derbyshire (n = 15), Kent (n = 58), London Metropolitan (n = 96), Merseyside (n = 44) and Sussex (n = 34) in 2004 / 5 (total n = 247). Reproduced from Potter, Clark, & Brown, (2008).

1.3.2 Self-medication

Substance abuse is highly prevalent in ADHD (Gudjonsson et al., 2012; Susan Young & Thome, 2011) (see Section 1.1.5). High rates of substance abuse has been found in those with undiagnosed ADHD compared to those without ADHD symptoms (Gudjonsson et al., 2012; Susan Young & Thome, 2011). This could suggest an attempt to self-medicate in those with undiagnosed ADHD (Bolea-Alamañac et al., 2014). In support of this, stimulant drugs are commonly diverted for cognitive enhancement as well as being abused, and alongside stimulants, cannabis is one of the most prominent drugs of abuse (Biederman et al., 1995). For example in a large epidemiological study (n = 10,987 adolescents) 5.4% met screening criteria for ADHD of which 21.1% vs 4.2% (non-ADHD) used marijuana and 12.1% vs 2.1% used amphetamines (Gudjonsson et al., 2012).

Figure 1-3 shows, in a prison population, significantly higher proportions of cannabis and stimulant use (but not alcohol or opiate use) were seen in prisoners who met clinical criteria for ADHD compared to prisoners who did not have ADHD (Asherson et al., currently unpublished data). In a large sample of adolescents (n=600) who either abused or were dependent on cannabis, 38% had ADHD, the second most common comorbidity (Dennis et al., 2004). Exclusion due to substance use disorder (SUD) was common in the OCEAN study in this thesis, with cannabis the most used drug (see Chapter 4, Section 4.2.2.5).

Research has found different motivations behind drug use in ADHD cases compared to controls. Adolescents with SUD who screened positive for ADHD were more likely to report improved self-image following drug use than were those with SUD without ADHD. The ADHD group were also more likely to report using drugs to alter their mood whereas the non-ADHD group to 'getting high' (Horner & Scheibe, 1997). This was corroborated in a separate study where young adults with ADHD were more likely than controls to report drug use in order to change their mood and improve sleep (Wilens, 2004). These differences in motivations could support the hypothesis of self-medication in ADHD.

Patients with ADHD in our studies (Chapters 5 and 6) report an improvement in behavioural symptoms when using cannabis. They report feeling reduced EL, restlessness and distractibility, with improved concentration, ability to sustain focus and sleep. Furthermore some patients report a preference for cannabis over stimulant medication in the treatment of their symptoms.

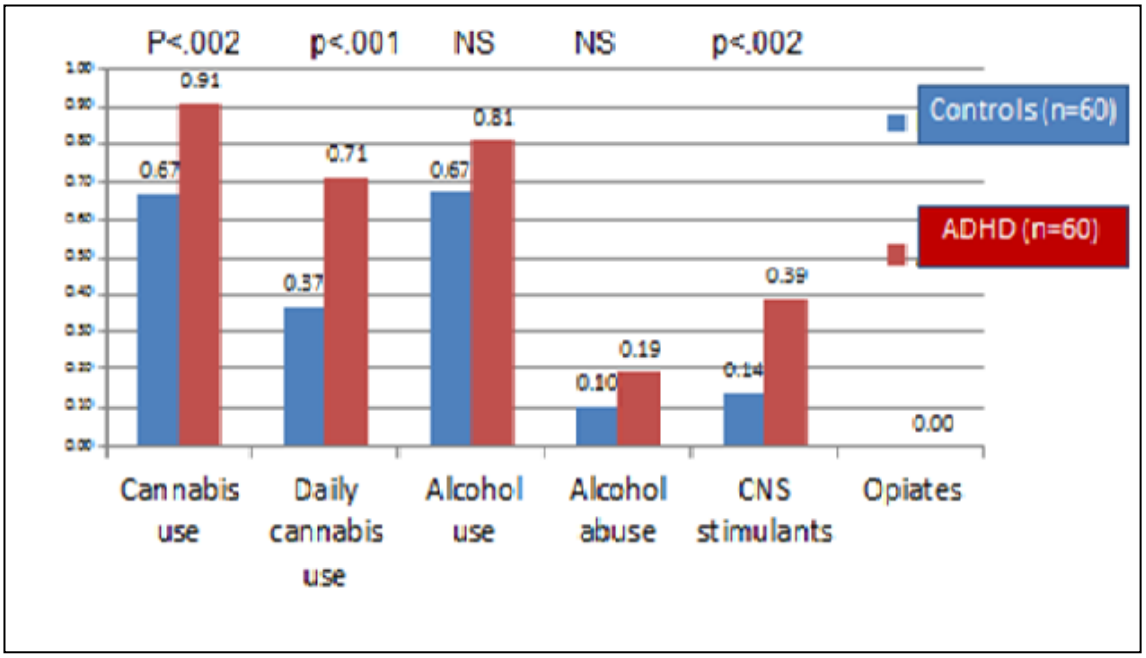

Figure 1-3: Proportion of drug and alcohol use in young offenders with and without ADHD (from the Concerta In Adult ADHD (CIAO) study, Asherson et al., currently unpublished data, reported at the World Congress of ADHD 2015)

1.3.3 Cannabis in the pathophysiology of ADHD

The dopamine theory of ADHD is well established, based largely on the immediate and rapid reduction of ADHD symptoms with stimulant medications (see Section 1.1.11). Children and adults with ADHD have dysfunctions that suggest the involvement of the dopaminergic circuits, mainly in the basal ganglia and frontal cortex, with deficits in executive function and in the reward system (see Section 1.1.9.4). The striatum is of particular interest in ADHD due to it being rich in dopaminergic synapses and neuroimaging studies have suggested reduced availability of dopamine in the striatum (Cheon et al., 2003; Dougherty et al., 1999; Krause et al., 2005; Krause et al., 2000; Spencer et al., 2005). Stimulant medication, the first line treatment in ADHD, acts to increase the synaptic availability of neurotransmitters, including noradrenaline and dopamine (Leonard et al., 2004; Volkow et al., 2002).

One way in which cannabinoids exert their effect on the mind is through interaction with the endogenous cannabinoid system (ECS) (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988). The ECS mediates the psychoactive effects of cannabis and refers to a set of endogenous ligands, their receptors, and the enzymes that synthesise and degrade them (Di Marzo, Bifulco, & De Petrocellis, 2004). Since striatal DA signalling may modulate the ECS and dopamine abnormalities have been found in ADHD, investigation of the ECS is of potential interest to our understanding of the processes that lead to the cognitive and behavioural dysfunctions seen in ADHD. A recent study investigating cannabinoid CB1 receptor (CB1R) function in a mouse model of ADHD obtained by triple point-mutation in the dopamine transporter (DAT) gene revealed that the sensitivity of cannabinoid receptors (CB1Rs) controlling GABA-mediated synaptic currents in the striatum was completely lost, and the mice had a marked hyperactive phenotype. These results point to a potential role of the ECS in neurobiological pathways underlying cognitive and behavioural deficits seen in ADHD (Castelli et al., 2011).

Although debated, there is evidence to suggest cannabis may affect dopamine production in brain areas implicated in ADHD. Animal studies have found administration of Δ9-THC to increase prefrontal dopamine levels (Chen, Paredes, Lowinson, & Gardner, 1990; Pistis et al., 2002). Human studies have found Δ9-THC administration to increase dopamine in the striatum in healthy volunteers (Bossong et al., 2009, 2015), and in patients with schizophrenia and their relatives

(Kuepper et al., 2013), with the latter finding the most pronounced release in the caudate nucleus. A PET study in healthy volunteers also found a significant increase in dopamine release following THC administration in the right middle frontal gyrus, left superior frontal gyrus and left superior temporal gyrus (Stokes et al., 2010). Although other studies in healthy volunteers have found no significant increase of dopamine in the striatum following administration of Δ9-THC (Barkus et al., 2011; Bloomfield et al., 2014; Stokes, Mehta, Curran, Breen, & Grasby, 2009).

Overlap between the action of THC and stimulant medication on the dopaminergic system have also been suggested (Piccini, Pavese, & Brooks, 2003; Stokes et al., 2010). Recent research using single photon emission computed tomography (SPECT) in participants with ADHD, ADHD and SUD, and healthy controls found no difference between striatal dopamine transporter density in controls and those with ADHD and SUD, whereas those with ADHD without SUD had significantly higher striatal dopamine transporter density than both groups. Higher density of dopamine transporters suggests lowered dopamine levels, suggesting self-medication in those with ADHD and SUD may act to normalise dopamine levels (Silva et al., 2013).

1.3.4 Cannabis and cognition

There is evidence to suggest cannabis impairs cognitive function. This may be due to the high to moderate densities of CB1 receptors in key areas of cognition, such as the hippocampus and frontal cortex (Glass, Dragunow, & Faull, 1997). Longitudinal studies have shown a significant decline in IQ in individuals who were heavy users of cannabis in adolescence (Meier et al., 2012; Mokrysz et al., 2014); although it is difficult to interpret any causal effects of cannabis use due to the possibility of confounding variables such as past history of other drug use, academic achievement and mood disorders (Schoeler & Bhattacharyya, 2013). For example, in one study the link between cannabis use and reduced IQ became non-significant after controlling for alcohol, tobacco, other drugs, gender, socioeconomic factors, maternal factors and mental health (Mokrysz et al., 2014). Research in typically developing participants suggests cannabis use leads to significant cognitive impairments including impairments in executive function, memory, sustained attention measured with omission errors), impulsivity (measured with commission errors), reaction time and motor function (Englund et al., 2012; Grant, Chamberlain, Schreiber, & Odlaug, 2012; Ramaekers et al.,

2006). However, this is not consistent with the subjective account of patients with ADHD, who may potentially show paradoxical benefits on brain function.

Furthermore, the degree of cognitive impairment may depend on the Δ9THC:CBD ratio as CBD may protect against impairment. In animal models of cognitive impairment CBD has been found to improve cognition (Avraham et al., 2011; Barichello et al., 2012; Fagherazzi et al., 2012; Magen et al., 2010), and protect against the memory impairing effects of Δ9-THC (Fadda, Robinson, Fratta, Pertwee, & Riedel, 2004). Studies in humans have also suggested CBD may cognitively protect against Δ9-THC induced impairments (Englund et al., 2012; Morgan, Schafer, Freeman, & Curran, 2010; Morgan et al., 2012). Research has also found participants who smoked cannabis higher in CBD to not show any impairments in immediate or delayed recall while intoxicated however participants who smoked cannabis with only Δ9-THC were significantly impaired (Morgan et al., 2010).

1.3.5 Sativex Oromucosal Spray: A case study of the prescription of a controlled cannabinoid medication

Sativex Oromucosal Spray (GW Pharma Ltd, Salisbury. UK), a standardised cannabinoid medication, was recently prescribed for a one month period to a patient at the Maudsley Adult ADHD Clinic. The reason was that the patient, who had a severe level of symptoms and impairments related to ADHD, found stimulants and atomoxetine to be ineffective, and even to exacerbate, his ADHD symptoms. Furthermore he had been using cannabis to control his ADHD symptoms with highly favourable accounts of an effective treatment response from both the patient and his mother. In this one case, treatment with Sativex resulted in improved control of ADHD symptoms, behaviour and cognitive function, reported by the patient and corroborated by his mother. The properties of Sativex are similar to cannabis resin (containing a 1:1 ratio of Δ9-THC:CBD).

1.3.6 Interim summary

Based on research and subjective patient accounts, self-medication with cannabis appears to be common in ADHD. Patients report improvements in concentration, restlessness, and EL. One theory of ADHD is that it is due to abnormalities in dopamine. A number of studies in animals and humans have found cannabis to increase dopamine production in brain areas implicated in ADHD. This has

suggested a potential role of the ECS in the neurobiological pathways underlying ADHD. The use of cannabis from the black market carries risk. In comparison to cannabis resin (which contains relatively equal Δ9-THC:CBD) sinsimella (which is high in Δ9-THC) has been linked to symptoms of psychosis and neurocognitive impairment. However it is becoming increasingly difficult to obtain cannabis resin. Therefore the investigation of a cannabinoid medication with a 1:1 ratio of Δ9-THC:CBD is of huge importance for the safety of patients.

1.4 Overall conclusions and aims of thesis

ADHD is a severe and impairing disorder that affects both children and adults. Although current pharmacological treatment has high levels of efficacy, some elect against this treatment due to adverse events, non-response, or an unwillingness to take pharmacological treatment. Two alternative treatments have been proposed. Omega-3 PUFA supplementation has been found to have a small to moderate benefit in reducing symptoms of ADHD in children. However the effects on cognition and EL remain unclear as do effects in adults with ADHD. Cannabis appears to be used to self-medicate by a number of adults with ADHD, some of whom report a preference for this over their stimulant medication. To our knowledge there has yet to be a controlled investigation of a cannabinoid medication in ADHD.

Aim 1: To document the effect of *n*-3 PUFA supplementation on cognition and emotional lability

In light of mixed findings, the first main aim of this thesis was to provide a more conclusive picture as to the effect of *n*-3 PUFA on cognition and EL. This was achieved in two ways. First, a systematic review and meta-analysis examining the effect of *n*-3 PUFA supplementation on cognition in healthy populations, and those with ADHD and related disorders, was conducted. Second, a systematic review and meta-analysis examining the effect of *n*-3 PUFA supplementation on EL, oppositional behaviour and conduct problems in children with ADHD and related disorders was conducted. The following hypothesis was tested:

- That supplementation with *n*-3 PUFA will improve cognition and EL.

Aim 2: The effect of *n*-3 PUFA supplementation in adults with ADHD: The OCEAN Study (Oils and Cognitive Effects in adult ADHD Neurodevelopment)

To the best of our knowledge a trial of *n*-3 PUFA supplementation in adults with ADHD is yet to be conducted. The second main aim of this thesis is to provide preliminary data on the relationship of *n*-3 PUFA to symptoms and cognition in adults with ADHD and controls. In order to do this a 6-month placebo-controlled trial of *n*-3 PUFA supplementation in 81 adults with ADHD was conducted, with 42 participants receiving the *n*-3 PUFA supplementation and 39 receiving the placebo. Outcome measures included cognitive and behavioural function. A group of 30 typically

developing controls additionally completed baseline assessments. The following hypotheses were tested:

- At baseline, we expected the ADHD group to have more severe ADHD symptoms, higher EL and impaired cognition, compared to the control group.
- We predicted reduced *n*-3 PUFA levels and an increased n-6:n-3 PUFA ratio in ADHD cases compared to controls.
- We expected supplementation with *n*-3 PUFA to improve ADHD symptoms, EL, and cognition.

Aim 3: The effects of Sativex on neurocognitive and behavioural function in adults with attention-deficit/hyperactivity disorder: the EMA-C study (Experimental Medicine in ADHD – Cannabinoids):

Despite the wide use of cannabis in ADHD and positive subjective patient reports, to our knowledge the effect of a cannabinoid based medication on cognitive and behavioural function in ADHD has yet to be tested. In line with this, the third main aim of this thesis is to provide preliminary data on the relationship of a short-term cannabinoid-based treatment (Sativex Oromucosal Spray) on symptoms and cognition in adults with ADHD. In order to do this, a 6-week double blind placebo controlled trial, with a group of 30 adults meeting research diagnostic criteria for ADHD was conducted. The group was divided into 15 participants who received Sativex and 15 who received the placebo. Outcome measures were cognitive performance, ADHD symptoms and EL. The following hypotheses were tested:

- That treatment with Sativex will improve cognitive performance.
- That treatment with Sativex will improve symptoms of ADHD and emotional lability.

Chapter 2: Omega-3 Polyunsaturated Fatty Acid Supplementation and Cognition: A Systematic Review and Meta-Analysis

Review

Omega-3 polyunsaturated fatty acid supplementation and cognition: A systematic review and meta-analysis

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Abstract

Background: Omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) are promoted as cognitive enhancers with consumption recommended in the general population and those with neurocognitive deficits such as attention deficit hyperactivity disorder (ADHD). However, evidence from randomised placebo-controlled trials is inconclusive.

Aims: This study aimed to conduct a systematic review and meta-analysis examining the effect of *n*-3 PUFA supplementation on cognition in healthy populations and those with ADHD and related disorders (RDs).

Methods: Databases were searched for randomised controlled trials (RCTs) in adults and school-aged children (who were healthy and typically developing (TD) or had ADHD or a related-neurodevelopmental disorder (ADHD+RD) which assessed the effects of *n*-3 PUFA on cognition.

Results: In the 24 included studies *n*-3 PUFA supplementation, in the whole sample and the TD and ADHD+RD subgroup, did not show improvements in any of the cognitive performance measures. In those with low *n*-3 PUFA status, supplementation improved short-term memory.

Conclusions: There is marginal evidence that *n*-3 PUFA supplementation effects cognition in those who are *n*-3 PUFA deficient. However, there is no evidence of an effect in the general population or those with neurodevelopmental disorders. This has important implications given the widespread advertisement and consumption of *n*-3 PUFA; claims of cognitive benefit should be narrowed.

Keywords

Attention deficit hyperactivity disorder, cognition, omega-3, randomised controlled trial, meta-analysis

Introduction

Global spending on omega-3 products is in the billions with consumption recommended in both the general population and those with neurocognitive deficits such as attention deficit hyperactivity disorder (ADHD; Bloch and Qawasmi, 2011), psychosis (Amminger et al., 2010), depression (Su et al., 2014) and autism (Yui et al., 2012). Stimulant medications significantly reduce the symptoms and cognitive impairments in ADHD (Banaschewski et al., 2006; Coghill et al., 2014; Faraone and Buitelaar, 2010). However some individuals elect against such medication due to undesirable side-effects, partial response and questions regarding the long-term efficacy and developmental effects (Dunnick and Hailey, 1995; Leonard et al., 2004; Nasrallah et al., 1986). Omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation is an extensively studied alternative treatment for ADHD, with meta-analyses of behavioural data demonstrating a small but significant effect on ADHD symptom improvement in children (Bloch and Qawasmi, 2011; Sonuga-Barke et al., 2013). It has also been proposed that *n*-3 PUFA supplements are important for the health of the brain and improve cognitive functions (Bryan et al., 2004). However, as yet there has been no systematic evaluation of the available evidence on which to draw any firm conclusions about its efficacy.

Longitudinal and cross sectional studies suggest an association between increased *n*-3 PUFA intake and cognitive function (Aberg et al., 2009; Bryan et al., 2004; Hibbeln et al., 2007). One of the main explanations proposed is based on the high lipid cell membrane composition, maintenance of which may be vital for the optimal development and function of the brain and nervous system (Bryan et al., 2004). However, randomised controlled trials (RCTs) in typically developing (TD) participants and those with ADHD and related neurodevelopmental disorders, have instead yielded mixed results. Benefits of *n*-3 PUFA supplementation on cognitive performance have been reported in healthy adults (Stonehouse et al., 2013) and children with ADHD (Sinn et al., 2008) and developmental coordination disorder (DCD) (Richardson and Montgomery, 2005). Yet a number of other studies in these populations have failed to find an effect (Jackson et al., 2012; Kairaluoma et al., 2009; Milte et al., 2012; Osendarp et al., 2007)

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Given the global market for omega-3 products it is of public importance that there is a more conclusive picture as to whether *n*-3 PUFA supplementation improves cognitive performance. We therefore conducted a systematic review and meta-analysis of randomised placebo-controlled trials which examined the effect of *n*-3 PUFA supplementation on cognitive performance in healthy populations and those with ADHD and related neurodevelopmental disorders.

Methods

We conducted a systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Eligibility criteria and data extraction

Studies were included if: (a) they were randomised double-blind placebo-controlled trials of *n*-3 PUFA supplementation including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linoleic acid (ALA). Trials supplementing with ALA alone were excluded as ALA is thought to have a limited impact on cognition compared to EPA and DHA (Kalmijn et al., 2004) and humans have a limited capacity to synthesise EPA and DHA from ALA (Goyens et al., 2005); (b) participants were school-aged children (4–12 years), adolescents (13–17 years) or adults (18–60 years) who were either healthy (TD group) or had a diagnosis of ADHD or high levels of ADHD symptoms or related neurodevelopmental traits such as DCD or dyslexia (ADHD+RD group); and (c) the study measured cognitive performance defined as (one or more measure of): intelligence quotient (IQ), inhibition, attention (omission errors), working memory, short-term memory, reading, spelling, mean reaction time and reaction time variability (see Supplementary Material, Table S1 for details). There were no language restrictions on trial eligibility.

The databases Ovid Medline (1946–September, week 2, 2014), Embase (1974–2014, week 37) and Psychinfo (1806–September, week 3, 2014) were searched. References of eligible trials and appropriate reviews were searched for additional citations. Unpublished or ongoing trials were searched on the ClinicalTrials.gov website and authors contacted to request relevant data. The search was updated in November 2014. The search terms used are listed in Supplementary Material, Table S2.

Risk of bias to determine study quality was assessed independently by two authors (REC and CT) according to PRISMA guidelines and the Cochrane Handbook of Systematic Reviews (Higgins and Green, 2011) (Supplementary Material, Table S3 and S4). Decision to include was based on risk of bias which was classed as low, unclear or high. Unresolved classification of studies was arbitrated by PA.

Data extraction was performed by REC and checked by a research assistant. The main outcome measures were the mean and standard deviation (SD) of the pre and post treatment cognitive performance measures for active and placebo arms, with intent to treat (ITT) analysis preferentially reported. Additional measures investigated included participant characteristics, study design and the supplement type and dose. If multiple treatment arms were present, only those supplementing with *n*-3 PUFA or placebo were included. With regard to missing data, we contacted authors. Missing data that remained unavailable was not imputed.

Cognitive performance measures

Nine domains of cognitive performance, previously found to be impaired in ADHD and related disorders (Doyle et al., 2005; Frazier et al., 2004; Kuntsi et al., 2009; Willcutt et al., 2010) were measured in these studies and included in this meta-analysis (see Figure 1). Examples of the main measures and tasks were as follows: IQ measured using the Wechsler Intelligence Scale for Children (WISC; Wechsler, 1991); commission errors (the inability to withhold a pre-potent response) on computerised tasks for inhibition (e.g. continuous performance tasks); omission errors (failing to respond when a response is required) on computerised attention tasks (e.g. test of variables of attention (TOVA) (Greenberg and Kindschi, 1996)) for attention (omission errors); digit span backwards (recalling a string of numbers backwards) for working memory; immediate or delayed word recall for short term memory; reading and spelling using subtests of the Wide Range Achievement Test (WRAT; Wilkinson and Roberts, 2006); mean reaction time (speed of responding) and reaction time variability (the variability in the speed of responding) during attention tasks (e.g. TOVA) (see Supplementary Material, Table S1 for a detailed list of cognitive measures).

Statistical analyses

Analyses were carried out in STATA (StataCorp, 2009) on the whole sample, the TD and ADHD+RD subgroups separately (with a further analysis of adults and children separately in the TD group) and then for the secondary subgroup analysis (see subgroup analysis section). Where a study contained two active groups which were both eligible for inclusion (for example when the active groups differed in the dose of *n*-3 PUFA), they were combined (with the method presented in the *Cochrane Handbook*: section 16.5.4; Higgins and Green, 2011). Effect sizes were estimated as the standardised mean difference (SMD); calculated as the mean pre-to post-treatment change, minus the mean pre-to post-placebo group change, divided by the pooled pre-test standard deviation (SD) with a bias adjustment (Morris, 2007). Effect sizes were classified according to Cohen's *d* (0.2=small, 0.5=medium, 0.8=large; Cohen, 1988). Where SD was not provided, it was calculated from sample size, *p*-values, *t*-values, standard error (SE) or 95% confidence intervals (CIs). For individual studies that contributed multiple assessments for one cognitive domain, a single SMD was derived from a meta-analysis of these assessments (see Supplementary Material, Table S1) hence an individual study was counted only once per cognitive domain. Cross-over trials were treated as parallel group trials using the pre-cross-over data, because insufficient data were provided to permit analysis of within-individual change (e.g. no correlations of scores between conditions). This approach is considered conservative (studies are under-rather than over-weighted) and is equivalent to setting the between-condition correlation to zero (Elbourne et al., 2002). SMDs in each domain were combined using the inverse-variance method where the reciprocal of their variance is used to weight the SMD

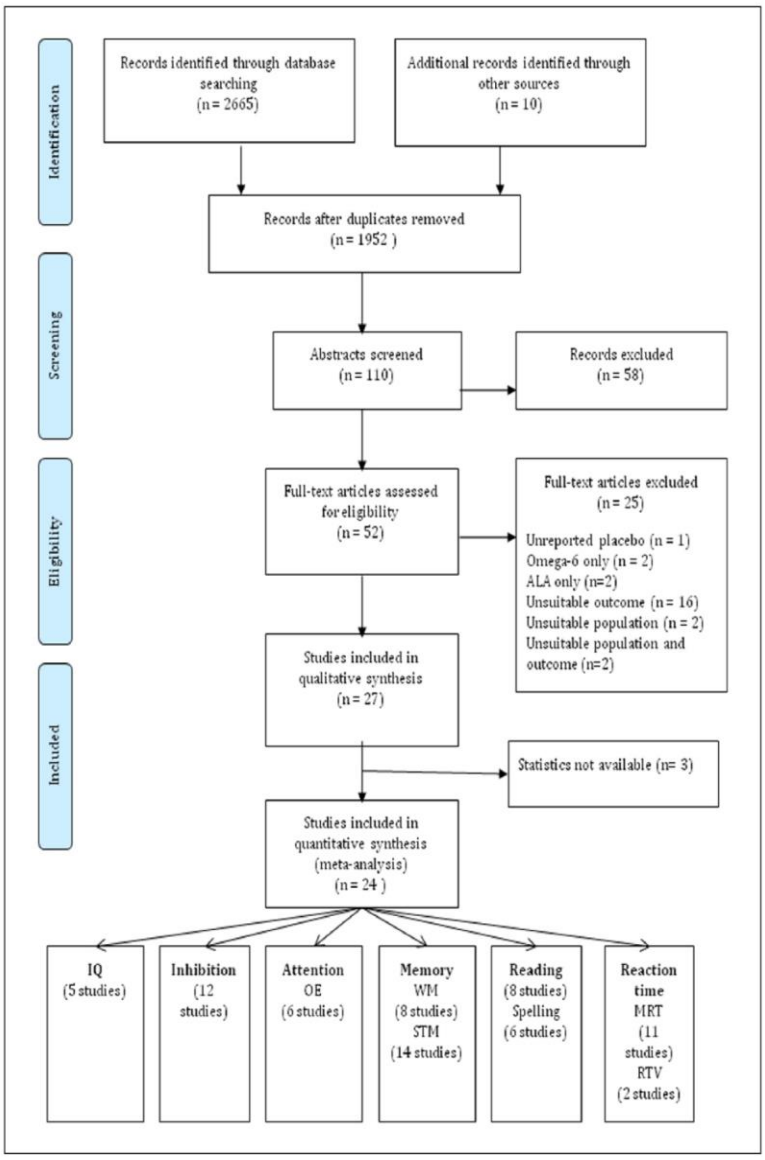


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.
ALA: alpha-linoleic acid; IQ: intelligence quotient; MRT: mean reaction time; OE: omission error; RTV: reaction time variability; STM: short term memory; WM: working memory.

from each trial before being combined to give an overall estimate (Higgins and Green, 2011). Given the between-study heterogeneity in terms of study design, participant characteristics and outcome measures, we chose a priori to use random effects models (Field and Gillett, 2010). When setting the significance level, we corrected for nine domains of cognition (Bonferroni correction set at $p<0.006$) despite the fact that the primary analysis was performed in the total sample and also separately for the ADHD+RD and TD groups (i.e. more than nine statistical tests were conducted), because the cognitive tests are highly correlated. The above p-value (0.006) was considered indicative and not evidence of association for the post-hoc analyses. A nominal level of significance was set at $p<0.05$. The I^2 statistic assessed heterogeneity between studies. Publication bias was assessed using the Egger regression asymmetry test (and inspection of the regression asymmetry plot) and the Begg adjusted rank correlation test. Meta-regression was used to examine the association between treatment effect and (a) trial duration and (b) dose of EPA and DHA. Four studies contained two active groups (Jackson et al., 2012; Kennedy et al., 2009; McNamara et al., 2010; Milte et al., 2012), therefore the average dose of EPA and DHA was taken across the two groups for the meta-regression and for the 'adequate EPA' subgroup analysis (see 'subgroup analyses' section).

Subgroup analyses

1. Strict inclusion: all studies that met our inclusion criteria were included in the primary analysis (as above). As two studies used supplementation with carnosine (Kairaluoma et al., 2009) or vitamins (Kirby et al., 2010) in addition to *n*-3 PUFA we performed subgroup analyses excluding these two studies.
2. PUFA deficient: it is proposed that only participants who are deficient in *n*-3 PUFA will benefit from treatment. The analysis was therefore run in four studies that supplemented: children of low socio-economic status who had low fish intake (defined in the paper as 'virtually no intake of fatty fish and a very low intake of lean fish,' Dalton et al., 2009, section 2.1), adults with low *n*-3 PUFA intake (less than ~200 mg EPA+DHA/wk, Stonehouse et al., 2013), malnourished children (53% consumed <1 portion fish a week, 39% one portion a week and 8% ⩾2 portions a week, Portillo-Reyes et al., 2014) and children with ADHD who were deficient in *n*-3 PUFA (participants were selected with thirst/skin problems indicative of *n*-3 PUFA deficiency, blood analysis showed these participants to have significantly lower *n*-3 PUFA compared with a TD control group, Stevens et al., 2003).
3. High quality: quality appraisal demonstrated the majority of studies to have design errors therefore the analysis was re-run in the eight studies whose overall risk score was low (and were therefore deemed high quality) (Supplementary Material, Table S3 and S4) (Jackson et al., 2012; Kairaluoma et al., 2009; Karr et al., 2012; Kennedy et al., 2009; Richardson and Montgomery, 2005; Richardson et al., 2012; Stonehouse et al., 2013; Vaisman et al., 2008).
4. Cognitive impairment: heterogeneity in cognitive impairments across study populations may reduce the effect size of treatment response. The analysis was run in four studies which included those with more homogenous cognitive deficits. Milte et al. (2012) tested children with ADHD whose literary performance was behind their year level at school. Vaisman et al. (2008) tested children with ADHD who also performed poorly on a continuous performance test. Richardson et al. (2012) tested a sub-group of the poorest readers (<20th centile from the total sample) and Kairaluoma et al. (2009) tested children with dyslexia.
5. Adequate EPA: a significant association between dose of EPA (but not DHA) and improvement in ADHD symptoms has previously been found (Bloch and Qawasmi, 2011). Given this, it has been suggested that EPA may be more active than DHA in terms of its effect on brain and behaviour. The analysis was therefore run in the 14 studies which supplemented participants with >100 mg EPA (this cut-off was estimated from Figure 3 in Bloch and Qawasmi's paper) (Antypa et al., 2009; Gustafsson et al., 2010; Hamazaki et al., 1996; Jackson et al., 2012; Kairaluoma et al., 2009; Karr et al., 2012; Milte et al., 2012; Parletta et al., 2013; Portillo-Reyes et al., 2014; Richardson and Montgomery, 2005; Sinn et al., 2008; Stonehouse et al., 2013; Vaisman et al., 2008; Widenhorn-Müller et al., 2014).

Results

Selection of studies

The search strategy (conducted by REC) identified 1952 publications. Of these, 110 relevant abstracts were screened, of which 58 were excluded because the studies were not an RCT (*n*=34), or they used an unsuitable outcome (e.g. looked only at treatment effects on PUFA blood levels) (*n*=10), population (*n*=12), supplement (*n*=1), or study design (*n*=1). Fifty-two full text articles were subsequently quality appraised (by REC and CT) and 25 excluded because of failure to report the placebo group (*n*=1), supplementation with omega-6 (*n*=2) or ALA (*n*=2) only, use of unsuitable outcome measures (*n*=16) (e.g. only measured behavioural outcomes), unsuitable population (*n*=2) or unsuitable outcome and population (*n*=2) (Supplementary Material, Table S5 lists the excluded studies). Of the 27 trials suitable for inclusion, after writing to the authors of studies with missing data, the statistical information required for meta-analysis was available for 24 studies, which made up the final dataset used in the meta-analysis (Figure 1 and Supplementary Material, Table S6).

Quality and characteristics of studies included in qualitative synthesis

Randomisation was explicitly described in 20 studies and allocation concealment in 17 studies. In the remainder this was absent or unclear. All studies were double blind apart from one, where the chief investigator was unblinded (although did not collect/analyse data) (Dalton et al., 2009). Inadequate allocation concealment in two studies meant participants were aware they were in different groups (Baumgartner et al., 2012; Dalton et al., 2009). Above-chance guessing (70%) of group allocation occurred in another study (Milte et al., 2012). Drop-outs (*n*=5/25) occurred only in the placebo group in one study (Portillo-Reyes et al., 2014). Reasons for drop-outs were not given in three studies (Benton et al., 2013; Kirby et al., 2010; Ryan and Nelson, 2008) despite one having more than double the amount of drop-outs in the active group (Ryan and Nelson, 2008). In one study the distribution of drop-outs between the placebo and active groups was not given (Gustafsson et al., 2010). In one study the *n*-3 PUFA supplements were taken only four days per week (Baumgartner et al., 2012). In two studies the active groups took supplementation with carnosine or vitamins in addition to *n*-3 PUFA (Kairaluoma et al., 2009; Kirby et al., 2010) (Supplementary Material, Table S3 and S4). Study characteristics are detailed in Supplementary Material, Table S7.

Quantitative meta-analysis

Of the 27 studies included in the qualitative synthesis, pre-and post-treatment means and SDs were not available for three studies (Hirayama et al., 2004; Long and Benton, 2013; Ryan and Nelson, 2008) therefore 24 studies were included in the meta-analysis. Omega-3 PUFA supplementation had no significant effect on any of the nine domains of cognitive performance in either the whole sample or the ADHD+RD or TD group (when analysed as a whole and by adults and children) separately. An effect on working memory in the ADHD+RD group approached significance (three studies, *n*=506) (SMD=0.23; 95% CI: –0.001–0.46, z=1.95, p=0.05) with no heterogeneity (x^2=3.03, I^2=33.9%,

Table 1. Main effects of meta-analysis for the whole sample (combined typically developing (TD), attention deficit hyperactivity disorder (ADHD)+related disorder (RD) group).

Domain	*n* studies	*n* participants	SMD	95% CI	Heterogeneity	
					p	I^2 (%)
IQ	5	434	0.14	−0.07–0.35	0.28	20.9
Inhibition	12	809	−0.04	−0.22–0.14	0.08	38.7
Attention (omission errors)	6	321	−0.13	−0.33–0.07	0.96	0.0
Memory (working memory)	8	1308	0.09	−0.01–0.18	0.40	3.9
Memory (short-term memory)	14	1914	0.07	−0.01–0.15	0.15	29.0
Reading	8	1579	0.02	−0.06–0.09	0.62	0.0
Spelling	6	1167	0.03	−0.09–0.15	0.39	5.0
Reaction time (mean reaction time)	11	1035	−0.002	−0.12–0.12	0.33	12.5
Reaction time (reaction time variability)	2	91	0.29	−0.70–1.28	0.02[a]	82.0

[a]Significant at $p<0.05$.

p=0.22). Main effects from the meta-analysis are summarised in Table 1 and Figure 2 and a detailed description of results is available in Supplementary Material, Section S1.

In the subgroup of those who were *n*-3 PUFA deficient a small treatment effect was found for short-term memory (three studies, n=331, SMD=0.26; 95% CI: 0.09–0.43, z=3.02, p=0.003) with no heterogeneity (x^2=2.67, I^2 =25.1%, p=0.26). In those who met strict inclusion criteria, a small treatment effect emerged for working memory after exclusion of one study which supplemented with vitamins (Kirby et al., 2010) (seven studies, n=960, SMD=0.15; 95% CI: 0.03–0.27, z=2.48, p=0.01) with no heterogeneity (x^2=4.18, I^2=0.0%, p=0.65). In studies that supplemented with adequate EPA a small treatment effect emerged for working memory (five studies, n=510, SMD=0.19; 95% CI: 0.04–0.34, z=2.44, p=0.02) with no heterogeneity (x^2=3.52, I^2=0.0%, p=0.47). Although the latter two treatment effects did not withstand correction for multiple testing (adjusted $p<0.006$). No other significant effects were found. Supplementary Material, Table S8 details the results of the subgroup analysis.

Significant heterogeneity was present in the TD group for IQ (x^2=4.12, I^2=75.8%, p=0.04) and short-term memory (x^2=17.66, I^2=49.0%, p=0.04). In the TD-child sample for inhibition (x^2=3.92, I^2=74.5%, p=0.05), in the TD-adult sample for mean reaction time (x^2=8.34, I^2=64.0%, p=0.04) and in ADHD+RD participants for reaction time variability (x^2=5.54, I^2=82.0%, p=0.02). In the sub-group analyses heterogeneity was found in those who were PUFA deficient for inhibition (x^2=8.16, I^2=75.5%, p=0.02) and mean reaction time (x^2=5.00, I^2=79.3%, p=0.03). Meta-regression found no effect of trial duration or dose of EPA or DHA on any of the eight domains of cognitive performance (there were not enough studies to examine this for reaction time variability (RTV)).

There was evidence of publication bias in working memory (Egger test only) (β=1.87, SE=0.58, t=3.23, p=0.02, 95% CI: 0.45–3.29) but not in any of the other eight domains of cognitive performance.

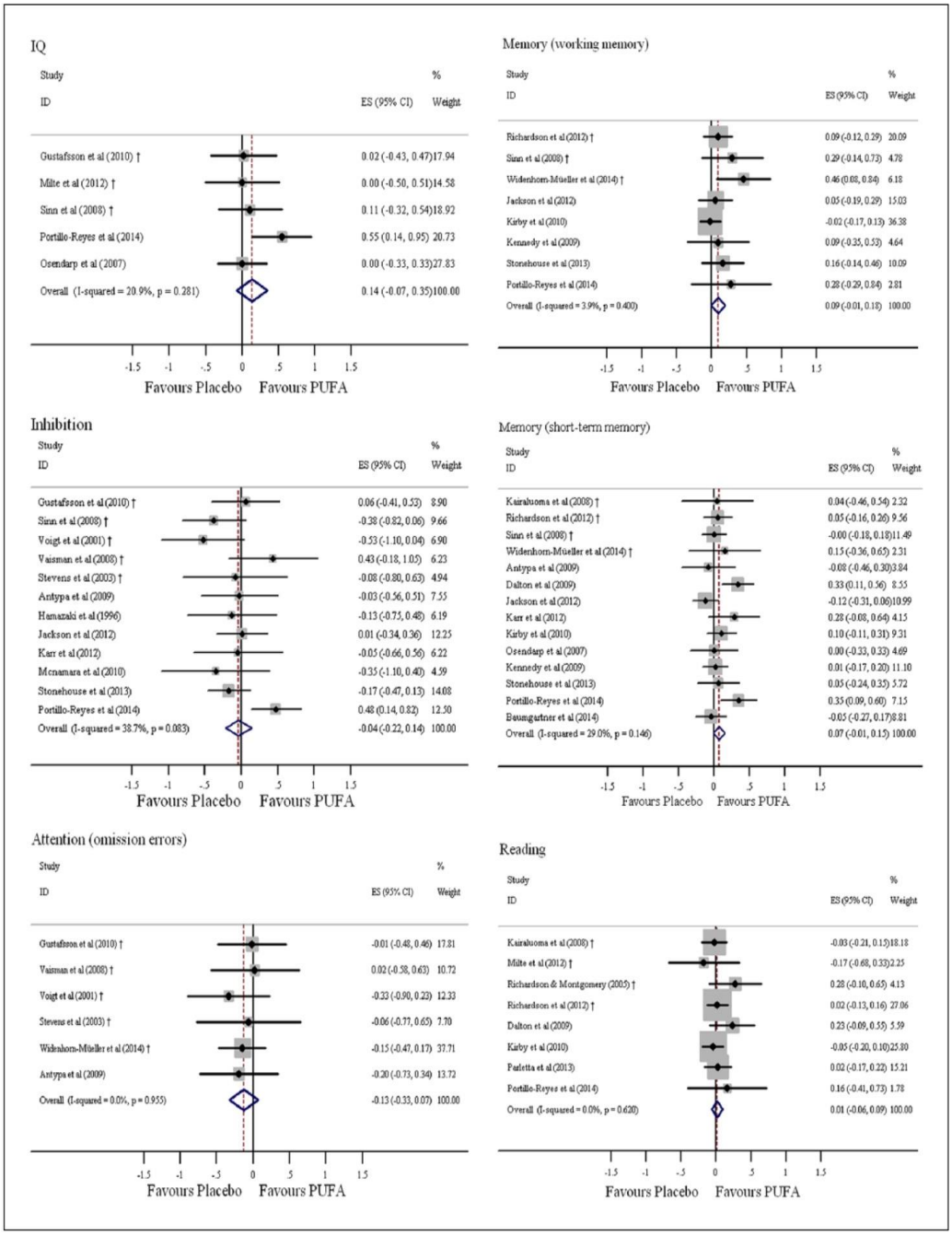
Discussion

This systematic review and meta-analyses examined the efficacy of *n*-3 PUFA supplementation on cognitive performance measures in school aged children and adults who were typically developing (TD) or had ADHD or a related neurodevelopmental disorder (ADHD+RD). We did not find an effect of *n*-3 PUFA supplementation on cognition in either the whole sample or the TD (analysed as a whole and by adults and children separately) or the ADHD+RD group when analysed separately. In the subgroup analyses a small treatment effect emerged for short-term memory in those with low *n*-3 PUFA and for working memory, after removal of a study which supplemented with vitamins (Kirby et al., 2010) and in those studies that supplemented with adequate EPA. However, both the effects on working memory were only nominally significant and were driven by the outcome of one cognitive measure in a small sample of 61 children with ADHD (Widenhorn-Müller et al., 2014).

There was no evidence of heterogeneity in the whole sample. Nominally significant heterogeneity was found in a number of sub-analyses (TD, ADHD+RD, TD-adult, TD-child, PUFA-deficient and high quality studies) this is most likely due to the smaller number of studies included in these analyses. Meta-regression found no effect of trial duration or EPA or DHA dose across any of the eight domains of cognitive performance (there were not enough studies to examine this for RTV). Evidence of publication bias was found only for working memory. We conclude on the basis of these data that there is no evidence of an effect of *n*-3 PUFA supplementation on cognitive performance in typically developing individuals or those with ADHD and related disorders. There is marginal evidence of benefit in those who are *n*-3 PUFA deficient. Evidence for those that met strict inclusion criteria or that supplemented with adequate EPA was much weaker.

A small improvement (which withstood correction for multiple testing) in short-term memory was found across four studies (in TD and ADHD+RD populations) which supplemented those with low *n*-3 PUFA (Dalton et al., 2009; Portillo-Reyes et al., 2014; Stevens et al., 2003; Stonehouse et al., 2013). Results from one study in malnourished children also found improvements in IQ following supplementation (Portillo-Reyes et al., 2014). This is in line with the suggestion that treatment effects on cognitive performance may occur only in those with low *n*-3 PUFA levels at baseline. However only four studies could be included in this subgroup (Dalton et al., 2009; Portillo-Reyes

et al., 2014; Stevens et al., 2003; Stonehouse et al., 2013) and whilst three of them measured PUFA-blood levels, only one of these examined blood-PUFA deficiency. Stevens et al., (2003) found reduced *n*-3 PUFA status in the ADHD study participants compared to TD controls. Therefore it cannot be certain that the other study participants were *n*-3 PUFA deficient. The subgroup analysis on those with low *n*-3 PUFA status found treatment effects in only one of the five cognitive performance domains. Therefore, although promising, further trials are needed before drawing any firm conclusions.



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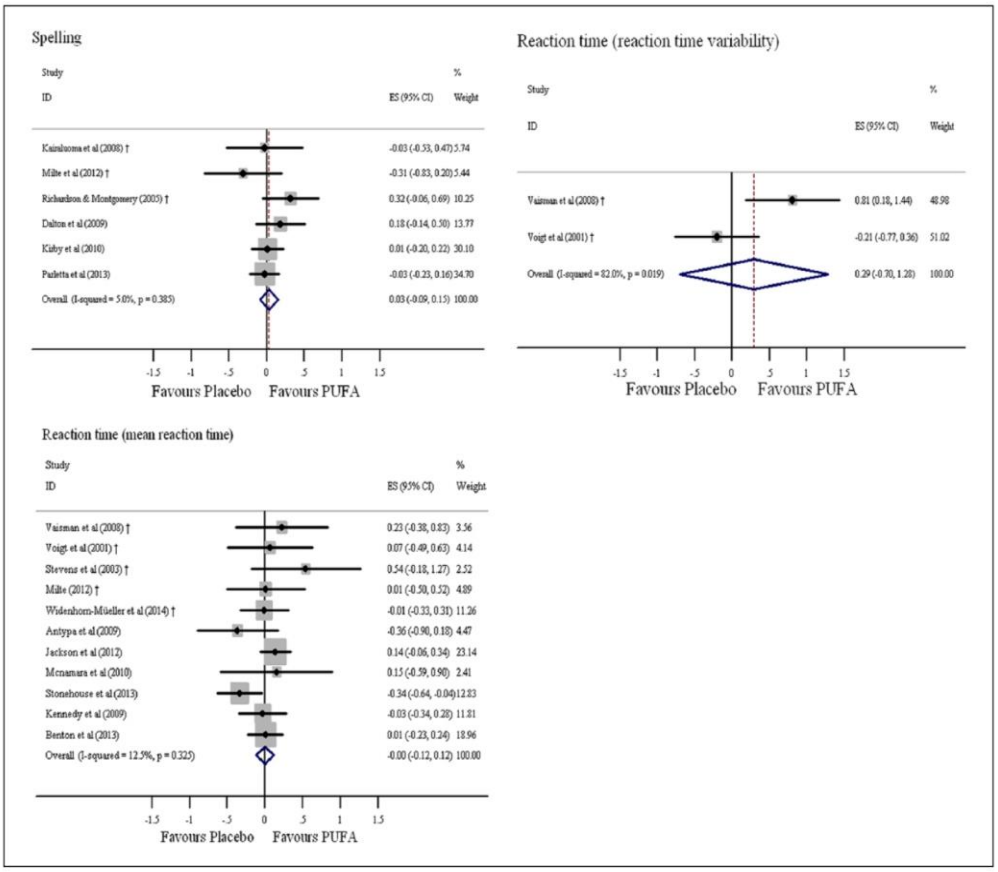

Figure 2. Forest plots for the meta-analyses in the whole sample across the nine domains of cognition. Studies without † indicates typically developing (TD) group. † indicates attention deficit hyperactivity disorder (ADHD)+ related disorder (RD) group.
CI: confidence interval; ES: effect size; ID: identification; IQ: intelligence quotient; PUFA: polyunsaturated fatty acid.

There was no evidence of an effect of *n*-3 PUFA supplementation in TD individuals and those with ADHD+RD. This is in line with the inconsistent findings from individual studies, with few positive findings remaining significant after correction for multiple testing (Antypa et al., 2009; Dalton et al., 2009; Hirayama et al., 2004; Jackson et al., 2012; Parletta et al., 2013; Sinn et al., 2008; Vaisman et al., 2008; Voigt et al., 2001). It is also in line with findings from the three studies included in the qualitative but not quantitative synthesis. Hirayama et al., (2004) found no effect on memory, attention or inhibition after eight weeks of supplementation in children with ADHD. Ryan and Nelson, (2008) found no effect on attention or inhibition after four months supplementation in healthy children. Long and Benton, (2013) found no effect on inhibition after three months supplementation in healthy adult males. This conclusion goes against previous narrative reviews which have suggested *n*-3 PUFA supplementation to improve cognitive performance (Assisi et al., 2006; Bryan et al., 2004; Horrocks and Yeo, 1999; Stonehouse et al., 2013). However, while these reviews highlighted interesting findings, they failed to provide a critical analysis in light of the mixed results on performance measures.

There are several important limitations to be considered before drawing conclusions. This study was limited by substantial between study variation with respect to patient groups, assessment procedures, outcome measures, treatment formulations, and quality in methods adopted for the different studies, necessitating the use of random effects models that produced wider confidence intervals. Due to reporting deficiencies the present study used pre-treatment SD instead of SD of the change (the difference before and after the intervention) in the calculation of effect size (Morris, 2007). This could have resulted in an underestimation of the true effect size (Ortego and Botella, 2010), although a sensitivity analysis of four studies of short-term memory which gave the SD of the change gave a similar, non-significant result (see Supplementary Material, Section S1).

In accord with our predominately negative findings, it has previously been suggested that treatment for ADHD may be more effective for the behavioural symptoms of inattention and hyperactivity-impulsivity, than cognitive performance measures (Coghill et al., 2014). In line with this, previous meta analyses have found a small but significant effect of *n*-3 PUFA supplementation on reducing ADHD symptoms in 699 (SMD=0.31, $p<0.0001$) (Bloch and Qawasmi, 2011) and 827 (SMD=0.21,

p=0.007) (Sonuga-Barke et al., 2013) children with ADHD. Furthermore, meta-analyses and systematic reviews have found a smaller treatment effect of stimulant medication on cognitive performance (~ 0.2–0.6) (Coghill et al., 2014) than on ADHD symptoms (~0.8-1.0) (Banaschewski et al., 2006; Faraone and Buitelaar, 2010). Several recent studies investigating the clinical response to methylphenidate found a dissociation of the treatment effects on ADHD symptoms and cognitive performance in children and adolescents with ADHD (Bédard et al., 2015; Coghill et al., 2007; Schulz et al., 2014). It is therefore suggested that different mechanisms are responsible for change in cognitive performance and change in behavioural symptoms (Coghill et al., 2007).

The lack of significant effects in the ADHD+RD group may reflect neuropsychological heterogeneity leading to a reduced effect size for individual domains of cognitive impairments, in comparison to ADHD symptoms where there is a more uniform deficit (Coghill et al., 2007; Nigg et al., 2005; Sonuga-Barke et al., 2010). For example, Vaisman et al., (2008) included children with a clinical ADHD diagnosis who also performed poorly on a continuous performance test and found a greater number of significant treatment effects on cognitive measures than studies that included those with a clinical ADHD diagnosis regardless of the baseline level of cognitive impairment (for example see Stevens et al., 2003). Although subgroup analyses across four studies which included those with more homogenous cognitive deficits (Kairaluoma et al., 2009; Milte et al., 2012; Richardson et al., 2012; Vaisman et al., 2008) failed to find treatment effects. However, given this small number of studies, further work would be required to test this specific sub-group hypotheses.

The studies used in this meta-analysis varied in supplement composition and dosage according to a previous meta-analysis higher EPA rather than DHA concentrations are associated with symptom reduction in children diagnosed with ADHD (Bloch and Qawasmi, 2011). However a subgroup analysis of those that supplemented with adequate (>100 mg) EPA and a meta-regression examining the relationship between EPA dose and cognitive task performance did little to support this. We found only one small (nominally significant) treatment effect for working memory which was driven by one study (Widenhorn-Müller et al., 2014) and no effect of EPA dose on cognitive performance.

There are inherent problems with blinding in studies which supplement with *n*-3 PUFA due to the fishy flavour of the capsules. The majority of studies did not assess blinding however above chance guessing occurred in one study that examined this (Milte et al., 2012). Identical flavouring of the placebo and active capsules must be used to reduce this limitation and the possibility of inflated effect sizes. A large number (*n*=7) of the studies included in the qualitative synthesis used an olive oil placebo. Olive oil contains a high concentration of oleic acid, a precursor of oleamide that has been shown to have psychoactive properties (Richardson, 2006). Stevens et al. (2003) found their olive oil placebo to be 'active' in that the supplement did not maintain the baseline PUFA composition. An inert substance such as liquid paraffin oil could be more suitable (Peet and Horrobin, 2002).

The majority of studies used in this meta-analysis were underpowered. The treatment effect that withstood correction for multiple testing (short-term memory in those who were *n*-3 PUFA deficient (SMD=0.26)) was small. With this modest effect size of around 0.3 we would require a sample size of around 352 participants (β=80%, two-tailed α=0.05) at a nominal level of significance and around 596 participants after correction for multiple testing (β=80%, two-tailed α=0.006). In the ADHD+RD group trials ranged from 40–362 participants with only three trials above 100. Although the largest trial (Richardson et al., 2012) in healthy children underperforming in reading found treatment effects on reading in only a subgroup of those who were the poorest readers and no effect on working memory. The largest trial in children with ADHD (*n*=110) (Widenhorn-Müller et al., 2014) again found only marginal evidence of a treatment effect with improvement in working memory but not in six other cognitive performance measures. Future studies should be adequately powered to detect small effects in order to clarify the presence of treatment effects.

We included only school-aged children and adults in our analysis (no trials in adolescent populations were located). The current results are therefore not generalisable to infants, adolescents or the elderly. Research has suggested similar negative results in these groups. A recent meta-analysis examined the effect of *n*-3 PUFA on cognitive performance in healthy elderly adults and those with cognitive decline. Across 10 domains of cognitive performance, treatment effects were found for those with cognitive decline in three domains (immediate and delayed recall, attention/processing speed). However significance was only at a nominal level (*p*=0.02–0.04) and became non-significant after correction for multiple testing (Mazereeuw et al., 2012). A Cochrane review and meta-analysis concluded RCTs in infants to have provided little evidence for the effect of *n*-3 PUFA on neurodevelopmental outcomes (including cognition) and inconsistent effects on visual acuity (Simmer et al., 2011).

Sex dimorphism may also be present in response to PUFA supplementation, thus analysis of samples as a whole and not by sex could potentially mask effects. One study found improvement in episodic memory in women and working memory in men (Stonehouse et al., 2013), potentially reflecting gender differences in problem-solving strategies. However, these findings were not corrected for multiple testing and further evidence would be required to examine the question of sex dimorphism in the cognitive response to *n*-3 PUFA supplementation.

Length of supplementation has also been proposed as a factor. In the current study only three trials were of six months or longer (Dalton et al., 2009; Osendarp et al., 2007; Stonehouse et al., 2013). Across two of these studies treatment effects were found on verbal learning ability, memory and reaction time (Dalton et al., 2009; Stonehouse et al., 2013). Although Stonehouse et al. (2013) tested a large number of cognitive domains, the majority of which were non-significant and failed to correct for multiple testing. The longest study (12 months) failed to find any treatment effects (albeit the dosage of *n*-3 PUFA was relatively small; Osendarp et al., 2007). The current study found no relationship between length of supplementation and effects on cognitive performance which is in line with a recent meta-analysis that found no relationship between trial duration and efficacy of *n*-3 PUFA supplementation in reducing ADHD symptoms (Bloch and Qawasmi, 2011). This evidence suggests that outcomes may have been uninfluenced by duration.

A number of outcome measures such as accuracy on cognitive tasks could not be included in this analysis due to the measures being too diverse to combine. However results from such measures were largely negative. For example treatment effects were not found in TD children (Kennedy et al., 2009) and adults (Jackson et al., 2012) for accuracy on reaction time tasks or in

children with ADHD for speed of information processing tasks (Widenhorn-Müller et al., 2014). Although one study (Sinn et al., 2008) found a significant benefit of treatment for accuracy on a sustained attention task, overall these results are in line with current negative findings.

In conclusion we have found no evidence of an effect of *n*-3 PUFA supplementation on cognitive performance in the general population or in those with ADHD and related disorders. There was suggestive evidence of improvements in those with low *n*-3 PUFA status. In order to provide a more conclusive picture future trials should employ larger sample sizes and should focus on supplementation of those who are *n*-3 PUFA deficient. It is suggested that regulators and producers of omega-3 products should consider this evidence when promoting their products.

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2.1 Chapter 2: Interim summary

Chapter 2 addressed part of the first aim of this thesis (see Section 1.4), to document the effect of *n*-3 PUFA supplementation on cognition. We found no evidence of a beneficial effect of *n*-3 PUFA on cognitive performance in the general population or in children with ADHD or with a related disorder. Marginal evidence for effects was found in those who were *n*-3 PUFA deficient. This evidence (Cooper et al., 2015) combined with research of the effects of *n*-3 PUFA on ADHD symptoms in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), provides us with the most accurate possible estimate of the effect of *n*-3 PUFA on ADHD symptoms (a small to moderate effect) and associated cognitive impairments (no effect aside from a potential (small) effect in those who are n-3 PUFA deficient) in children with ADHD. I will now address the remainder of the first aim of this thesis: to document the effect of *n*-3 PUFA supplementation on emotional lability, a characteristic feature of ADHD.

Chapter 3: The Effect of Omega-3 Polyunsaturated Fatty Acid Supplementation on Emotional Lability, Oppositional Behaviour and Conduct Problems in ADHD: A Systematic Review and Meta-Analysis

3.1 Summary

A number of randomised controlled trials report a beneficial effect of omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation on emotional lability (EL) and related domains (e.g. oppositional behaviour, conduct problems). Given that *n*-3 PUFA supplementation shows a significant effect on reducing symptoms of attention-deficit/hyperactivity disorder (ADHD) and that EL and related behaviours commonly co-occurs with ADHD, it is important that there is a more conclusive picture as to the effect of *n*-3 PUFA on these co-occurring clinical domains. The databases (Ovid Medline, Embase, Psychinfo) were searched for trials assessing the effects of *n*-3 PUFA on EL, oppositional behaviour, aggression and conduct problems. We included trials in children who had ADHD or a related neurodevelopmental disorder. Of the 1775 identified studies, 10 were included in the meta-analysis. In the primary analyses *n*-3 PUFA supplementation did not show improvements in measures of EL, oppositional behaviour, conduct problems or aggression. However subgroup analyses of higher quality studies and those meeting strict inclusion criteria found a significant reduction in EL and oppositional behaviour. A number of treatment effects may have failed to reach statistical significance due to small sample sizes and within and between study heterogeneity in terms of design and study participants. These results exclude the possibility of moderate to large effects. They provide suggestive evidence of small effects of *n*-3 PUFA on reducing EL and oppositional behaviour in subgroups of children with ADHD.

3.2 Introduction

Omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation is one of the most studied alternative treatments for ADHD (Bloch & Qawasmi, 2011). Two recent meta-analyses of

randomised placebo-controlled trials have found *n*-3 PUFA supplementation to have a small but significant effect of reducing ADHD symptoms of inattention and hyperactivity/impulsivity (Bloch & Qawasmi, 2011; Sonuga-Barke et al., 2013). The DSM-5 also lists emotional lability (EL; defined as low frustration tolerance, irritability and mood lability) and cognitive impairment (defined as problems on tests of attention, executive function or memory) as associated features of ADHD that support the diagnosis of ADHD (American Psychiatric Association (2013). We recently found little evidence for an effect of *n*-3 PUFA on cognition in children with ADHD or a related-neurodevelopmental disorder (Cooper et al., 2015). Emotional lability, characterised by irritable moods with volatile and changeable emotions, relates to the common co-occurrence of conduct, oppositional and emotional behaviour problems found in ADHD (Bolea-Alamañac et al., 2014). However the response of EL to *n*-3 PUFA is not yet clear.

Deficiency in *n*-3 PUFA status has been associated with EL. Prenatal and childhood deficiency of *n*-3 PUFA may impair neuronal migration, connectivity, timed apoptosis (cell death) and dendritic arborization (neuronal branching), leading to disruption of the neuronal pathways that regulate behaviour (for review see Hibbeln et al., 2006). Deficiencies may also result in altered serotonin and dopamine levels (Assisi et al., 2006; Chalon, 2006; Haag, 2003; Hibbeln et al., 2006; Young & Conquer, 2005); neurotransmitters implicated in the pathophysiology of ADHD (Bolea-Alamañac et al., 2014; del Campo et al., 2012; Faraone et al., 2005; Gizer et al., 2009; Li et al., 2006). Low concentrations of serotonin have been related to impulsive, violent, suicidal, hostile and aggressive behaviours (Hallahan & Garland, 2004; Hibbeln et al., 1998).

Epidemiological and cross-sectional studies have linked violent behaviour with low seafood consumption or low blood *n*-3 PUFA levels (Corrigan et al., 1994; Hibbeln, 2001; Iribarren et al., 2004). In children and adolescents with ADHD and symptoms of conduct-disorder low omega-3 blood levels were found to be negatively related to high scores of callous and unemotional traits (Gow et al., 2013). A greater number of behaviour problems and temper tantrums were found in children (with and without ADHD) with lower total *n*-3 PUFA blood levels (Stevens, Zentall, Abate, Kuczek, & Burgess, 1996).

However, evidence from trial data of *n*-3 PUFA supplementation has been mixed. One of the most prominent findings was from a trial conducted in a prison. Gesch et al., (2002) supplemented a total of 231 prison inmates with PUFAs (omega-3 and 6), vitamins and minerals or placebo. A 26.3% reduction in disciplinary offences was found in the active versus the placebo group ($p< 0.03$). Meta-analysis of eight placebo-controlled trials in healthy populations and those with various mental health conditions found aggression to be reduced in those taking *n*-3 PUFA supplements (Benton, 2007). However, this meta-analysis had several limitations, including the combination of a range of heterogeneous measures and study populations, and 5 of the 8 studies were from the same research group (Hamazaki et al., 1998, 2002; Hamazaki et al., 1996; Hirayama, Hamazaki, & Terasawa, 2004; Itomura et al., 2005). Results from placebo-controlled trials, in those with ADHD or who had overlapping neurodevelopmental disorders (such as specific reading difficulties), have been more variable, with some finding improvements on rating scale measures of EL and oppositional behaviour (Richardson et al., 2012; Richardson & Montgomery, 2005; Stevens et al., 2003), while other studies failed to find such effects (Manor et al., 2012; Milte et al., 2012; Widenhorn-Müller et al., 2014).

Given the finding of a small but significant effect of *n*-3 PUFA on ADHD with an effect size (Cohen's *d*) in the region of 0.2–0.3 (Bloch & Qawasmi, 2011; Sonuga-Barke et al., 2013), yet mixed findings on associated comorbid symptoms of EL and oppositional behaviour, it is important to clarify whether there is a role for *n*-3 PUFA in the management of these associated features. To answer this question we conducted a systematic review and meta-analysis of randomised placebo-controlled trials which examined the effect of *n*-3 PUFA supplementation on EL, oppositional behaviour, conduct problems, and aggression in children with ADHD and related neurodevelopmental disorders (ADHD+RND).

3.3 Methods

We conducted a systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009) and used a predetermined protocol. We considered the analyses to be exploratory; assessing effects on six separate domains: of parent rated EL, teacher rated EL, parent rated

oppositional behaviour, teacher rated oppositional behaviour, parent rated conduct problems and parent-rated aggression (teacher-ratings were not available for conduct problems or aggression) .Parent and teacher ratings were considered separate due to the established discrepancy between these measures (Goodman, 1997).

3.3.1 Eligibility criteria and data extraction

Studies were included if: 1) they were randomised double blind placebo-controlled trials of *n*-3 PUFA supplementation including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) or alpha-linoleic acid (ALA; although those supplementing with ALA only were excluded as ALA is thought to have limited impact on behaviour compared to DHA and EPA (Kalmijn et al., 2004); 2) Participants were school aged children (4-12 years), adolescents (13-17 years), or adults (18-55 years) who had a diagnosis of ADHD, had high levels of ADHD-symptoms or related neurodevelopmental traits such as developmental coordination disorder; and 3) the study contained at least one outcome measure which targeted EL, oppositional behaviour, conduct problems or aggression using a validated rating scale (see Appendix B, Table AB-1). There were no language restrictions on trial eligibility.

The databases Ovid Medline (1946 to September week 3 2014), Embase (1974 to 2014 September 29th), and Psychinfo (1806 to September week 4 2014) were searched. References of eligible trials and appropriate reviews were searched for additional citations. Unpublished or ongoing trials were searched on the ClinicalTrials.gov website and authors contacted to request relevant data. The search was updated in January 2015. The search terms used are listed in Appendix B, Table AB-2.

Risk of bias to determine study quality was assessed independently by two authors (REC and CT) according to PRISMA guidelines and the Cochrane Handbook of Systematic Reviews (Higgins & Green, 2011) (see Appendix B, Table AB-3 and AB-4). REC and CT then met to discuss their assessments and reach a consensus on study inclusion. Decision to include was based on risk of bias (classed as low, unclear or high); those which were classed as high overall risk of bias were excluded.

Data extraction was performed by REC and checked by a research assistant. The main outcome measures were the mean and standard deviation (SD) of the pre-and post-treatment measures of EL, oppositional behaviour, conduct problems and aggression for active and placebo arms, with

intent to treat (ITT) analysis preferentially reported. Additional measures investigated participant characteristics, study design, and the type and dose of the supplement used. If multiple treatment arms were present, only those supplementing with *n*-3 PUFA or placebo were included. With regard to missing data, we contacted authors. Missing data that remained unavailable was not imputed.

3.3.2 Statistical Analyses

Analyses were carried out in STATA (StataCorp, 2009). An initial analyses in the full sample across the six domains of parent and teacher rated oppositional behaviour and emotional lability, teacher rated conduct problems and aggression was first run, following this four subgroup analyses (discussed below) were conducted. Where a study contained two active groups which were both eligible for inclusion (for example when the active groups differed in the dose of *n*-3 PUFA), they were combined with the method presented in the Cochrane handbook: Section 16.5.4 (Higgins & Green, 2011). Effect sizes were estimated as the standardised mean difference (SMD), calculated as the mean pre-to-post-treatment change, minus the mean pre-to-post-placebo group change, divided by the pooled pre-test standard deviation (SD) with a bias adjustment (see Morris, (2007), pg 369 'effect size estimate using pooled pretest SD' for a detailed description of this method). The equation for this method is detailed below.

$$d_{ppc2} = c_p \left[\frac{\left(M_{post,T} - M_{pre,T}\right) - \left(M_{post,C} - M_{pre,C}\right)}{SD_{pre}} \right]$$

$$SD_{pre} = \sqrt{\frac{(n_T - 1)SD_{pre,T}^2 + (n_C - 1)SD_{pre,C}^2}{n_T + n_C - 2}}$$

$$C_p = 1 - \frac{3}{4(n_T + n_C - 2) - 1}$$

Note. d_{ppc2} = Standardised Mean Difference (SMD), C_p = bias adjustment, M = Mean, T = treatment, C = Control, Post = Post-treatment, Pre = Pre-treatment, SD = Standard deviation, n = number of participants.

Effect sizes were classed according to Cohen's *d* (0.2=small, 0.5=medium, 0.8=large) (Cohen, 1992). Where SD was not provided, it was calculated from sample size and standard error (SE). For

individual studies that contributed multiple assessments for one domain, a single SMD was derived from a meta-analysis of these assessments (see Appendix B, Table AB-1); hence an individual study was counted only once per domain. Cross-over trials were treated as parallel group trials using the pre-cross over data, because insufficient data were provided to permit analysis of within-individual change (i.e. correlations of scores between conditions were not given). This approach is considered conservative (studies are under-rather than over-weighted), and is equivalent to setting the between-condition correlation to zero (Elbourne et al., 2002). SMDs in each domain were combined using the inverse variance method where the reciprocal of their variance is used to weight the SMD from each trial before being combined to give an overall estimate (Higgins & Green, 2011).

Given the between-study heterogeneity in terms of study design, participant characteristics and outcome measures, we chose a priori to use random effects models (Field & Gillett, 2010). When setting the significance level, we corrected for 6 domains (see 'selection of studies') of emotional/behavioural instability (Bonferroni correction set at $p < .008$). This was despite the fact that more than 6 statistical tests were conducted (i.e. four subgroups containing multiple analyses were also conducted), because the 6 behavioural domains are highly correlated. The significance level of $p<.008$ was therefore considered indicative and not evidence of association for the sub-group analyses (this is similar to our method used in Cooper et al., (2015). A nominal level of significance was set at $p<.05$. The I^2 statistic assessed heterogeneity between studies. Publication bias was assessed using the Egger regression asymmetry test, inspection of the regression asymmetry plot and the Begg adjusted rank correlation test. Metaregression was used to examine the association between treatment effect and trial duration and dose of EPA and DHA.

3.3.3 Subgroup analyses

1) Strict inclusion: All studies that met the inclusion criteria were included in the initial analysis. As one study supplemented with the phospholipid molecule phosphatidylserine (Manor et al., 2012) in addition to *n*-3 PUFA, we performed subgroup analyses excluding this study.

2) High quality: Quality appraisal demonstrated the majority of studies included in the main analyses to contain design errors (overall risk of bias rated as unclear or unclear to high). Therefore the analysis was re-run in the four studies whose overall risk scores were low (equating to high

quality) (Manor et al., 2012; Richardson et al., 2012; Richardson & Montgomery, 2005; Richardson & Puri, 2002) (see Appendix B, Table AB-3 and AB-4).

3) Adequate EPA: A significant association between dose of EPA (but not DHA) and improvement in ADHD symptoms has previously been found (Bloch & Qawasmi, 2011). Given this, it has been suggested that EPA may be more active than DHA in terms of its effect on brain and behaviour. The analysis was therefore run in the seven studies which supplemented participants with > 100mg EPA (Dean, Bor, Adam, Bowling, & Bellgrove, 2014; Gustafsson et al., 2010; Milte et al., 2012; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Sinn & Bryan, 2007; Widenhorn-Müller et al., 2014) (this cut-off was estimated from Figure 3 in Bloch and Qawasmi's (2011) paper).

4) High EL and related domains: Heterogeneity in EL and its related domains, across study populations, may reduce the effect size of treatment response. The analysis was run in 2 studies that included those with elevated impairments in these domains. Gustafsson et al., (2010) analysed a subgroup of children who had oppositional problems at a clinical level and Manor et al., (2012) analysed a subgroup of children who were more labile and hyperactive, and who tended to suffer from mood and behavioural dysregulation.

3.4 Results

3.4.1 Selection of studies

The search strategy (conducted by REC) identified 1775 publications. Of these, 149 relevant abstracts were screened, of which 97 were excluded because the studies were not a randomised clinical trial (N=40); or they used an unsuitable outcome (N=28) (e.g. looked only at treatment effects on PUFA blood levels), study population (N=23) or supplement (N=6). Fifty-two full text articles were subsequently quality appraised and 40 excluded because of failure to report the placebo group (N=1), unsuitable supplementation (N=4), unsuitable populations (N=11), or use of unsuitable outcome measures (N=24) (see Appendix B, Table AB-5). Twelve trials met the inclusion criteria for this study; however, due to missing data (after writing to the authors), the statistical information required for meta-analysis was available for 10 of the studies which made up the final dataset for the meta-analysis. Of these 10 studies, 7 were in those who met diagnostic criteria for ADHD or were selected for high levels of ADHD symptoms and 3 were in those with a

developmental disorder known to overlap with ADHD (developmental coordination disorder, disruptive behaviour disorder and reading difficulties) (Figure 3-1 and Appendix B, Table AB-6).

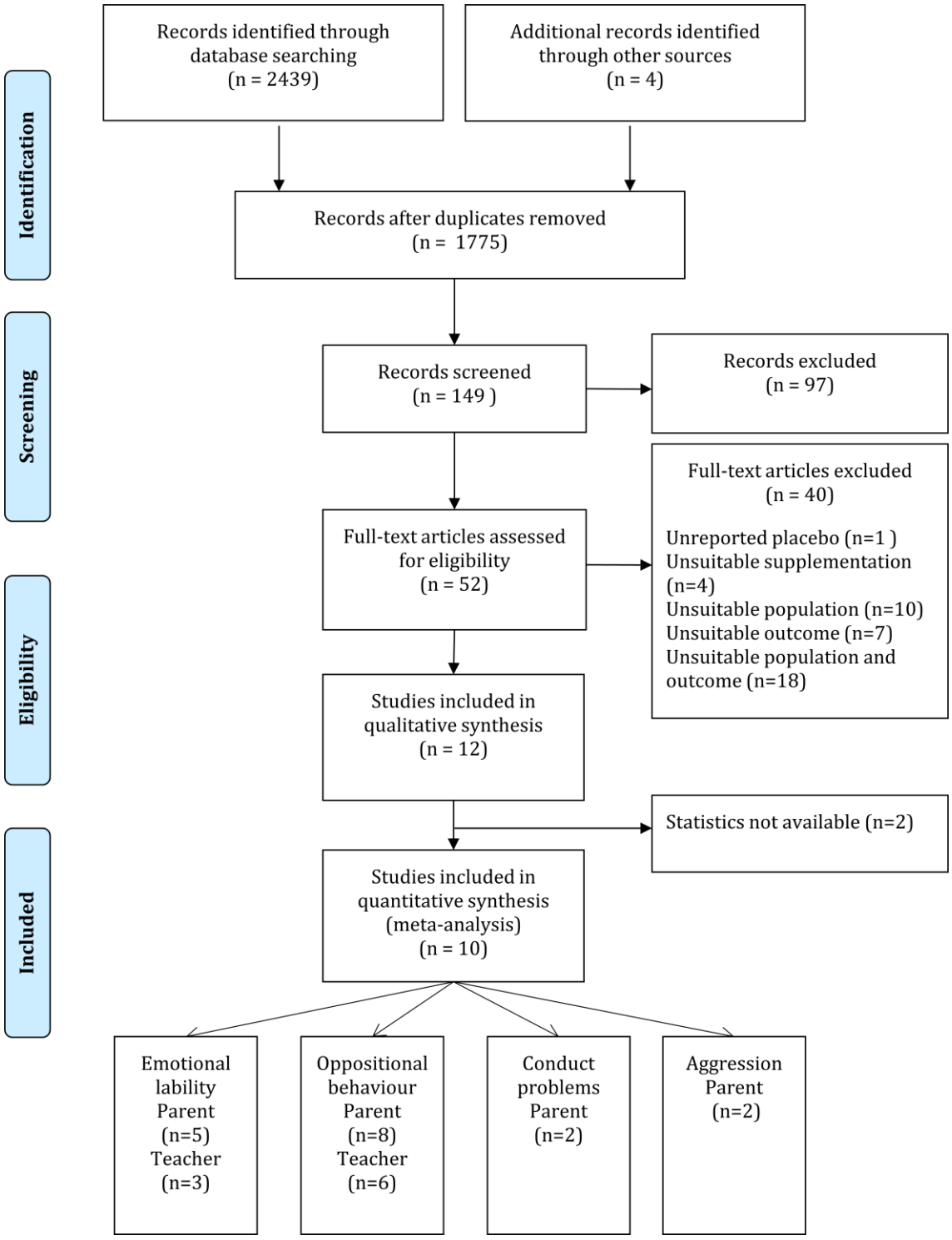


Figure 3-1: PRISMA flow diagram

3.4.2 Outcome measures

Six outcome domains were included in the meta-analysis (parent-rated EL, teacher-rated EL, parent-rated oppositional behaviour, teacher-rated oppositional behaviour, parent-rated conduct problems and aggression). EL, oppositional behaviour and conduct problems were mainly

measured using the parent-or teacher-rated Conners' scales (Conners, 1990), or the Strengths and Difficulties Questionnaire (Goodman, 1997). Aggression was parent-rated and was measured using various rating scales; the Child Behaviour Checklist (CBCL) (Arbeitsgruppe Deutsche Child Behavior Checklist, 1993a), Modified Overt Aggression Scale (MOAS) (Connor, 2002) or the Children's Aggression Scale (CAS) (Halperin, McKay, & Newcorn, 2002). Appendix B, Table AB-1 contains a detailed list of measures used in these six domains.

3.4.3 Quality and characteristics of studies included in qualitative synthesis

Study quality was assessed independently by two authors (REC and CT). Twelve studies were agreed to be of sufficient quality and suitability and were included in the qualitative synthesis. As there were no studies in adolescents or adults, the qualitative synthesis was limited to school-aged children (4-12). Randomisation and allocation concealment were explicitly described in 10 studies. In the remainder this was absent or unclear. All studies were double blind, although above chance guessing of group allocation occurred in one study (Milte et al., 2012). In one study 18% dropped out because their parents wanted pharmacotherapy and it is unclear whether this was equally distributed between the placebo and active groups (Gustafsson et al., 2010). Four studies did not report the appropriate statistics (Bélanger et al., 2009; Gustafsson et al., 2010; Hirayama et al., 2004; Widenhorn-Müller et al., 2014). For example, one study reported only medians (Hirayama et al., 2004) and another failed to report means and SDs for 2 of the 3 outcome variables (Widenhorn-Müller et al., 2014) (see Appendix B, Table AB-3 and AB-4). One study used supplementation with phosphatidylserine (a phospholipid component) (Manor et al., 2012) in addition to *n*-3 PUFA. Children were unmedicated in nine studies, medicated in two studies and in one study this was unspecified. Study characteristics are detailed in Appendix B, Table AB-7.

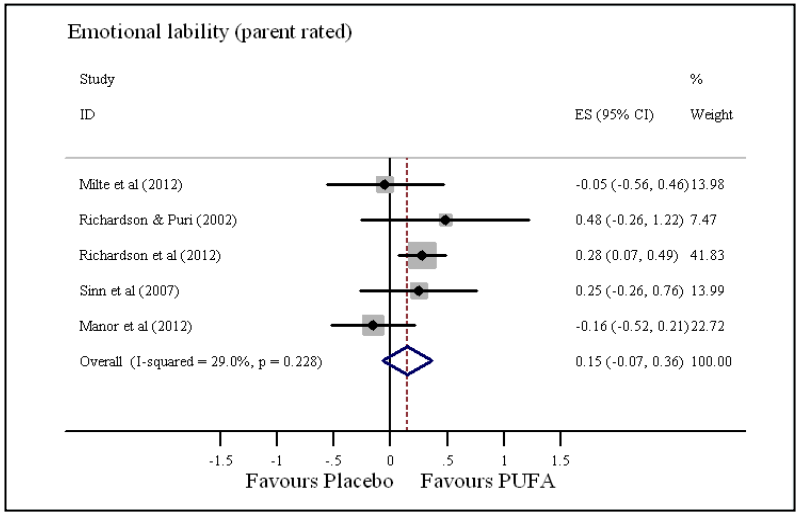
3.4.4 Quantitative meta-analysis

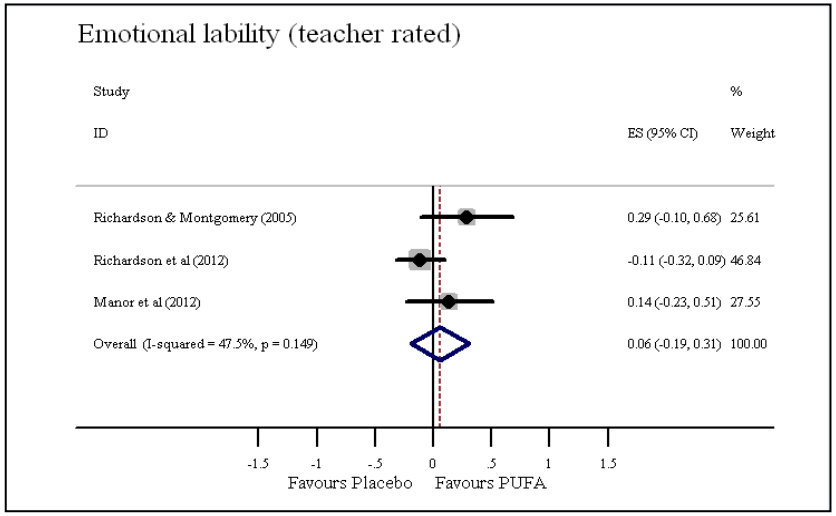
Main effects from the meta-analysis are summarised in Figure 3-2, a detailed description of these results is available in Appendix B, Supplement AB-1. Pre-and post-treatment means and SDs were not available for two studies (Bélanger et al., 2009; Hirayama et al., 2004). Therefore 10 studies were included in the meta-analysis.

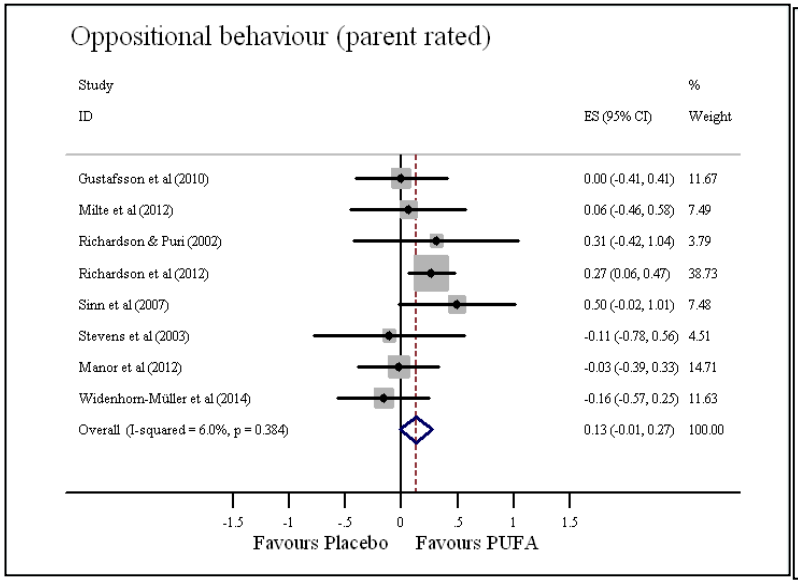
In children with ADHD+RND *n*-3 PUFA supplementation had no significant effect on parent- and teacher-rated symptoms of EL or oppositional behaviour, parent-rated conduct problems, and no effect on aggression (although there was a trend for supplementation to improve parent rated oppositional behaviour (8 studies, N=875, SMD = 0.13; 95% CI: -0.01 to 0.27, z =1.77, p = 0.08)). There was no evidence of heterogeneity. There was no evidence of publication bias (the Egger test could not be run for parent rated conduct problems or aggression due to too few studies). Meta-regression found no effect of treatment duration or dose of EPA or DHA (meta-regression could not be run for duration or dose (EPA or DHA) for aggression and parent rated conduct problems and EPA dose for teacher rated EL due to there being too few studies).

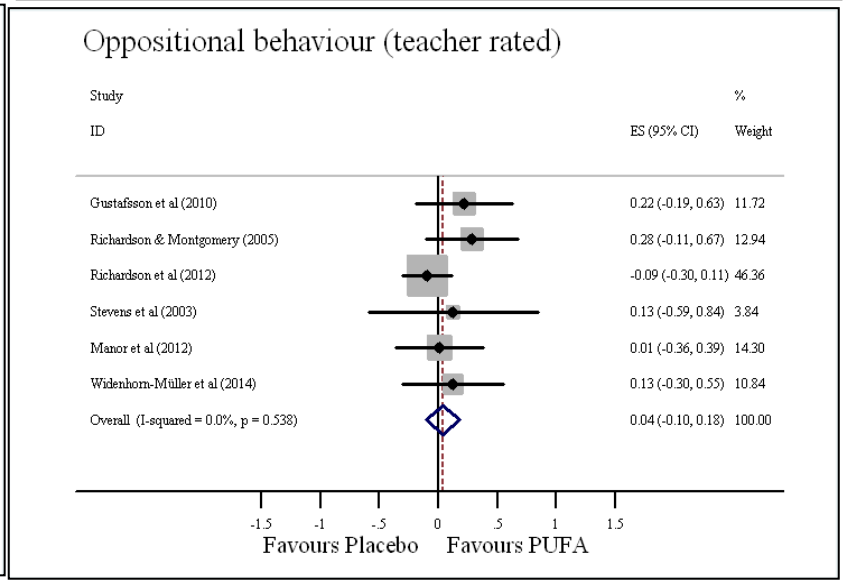
Sub-group analyses: In the subgroup analysis of studies that met strict inclusion criteria, a small treatment effect was found for parent-rated EL after exclusion of one study which supplemented with *n*-3 PUFA and phosphatidylserine (4 studies, 515 participants, SMD = 0.25; 95% CI: 0.08 to 0.43, z = 2.81, p = 0.005), with no evidence of heterogeneity (x^2 = 1.78, I^2 = 0.0%, p = 0.62). In the subgroup of high quality studies a small treatment effect was found for parent-rated oppositional behaviour (3 studies, 532 participants, SMD = 0.20; 95% CI: 0.03 to 0.38, z = 2.26, p = 0.02), with no heterogeneity (x^2 =2.00, I^2 =0.2%, p = 0.37). There was a trend for supplementation to improve parent-rated oppositional behaviour after exclusion of one study which supplemented with *n*-3 PUFA and phosphatidylserine (7 studies, N=734, SMD = 0.15; 95% CI: -0.006 to 0.31, z =1.89, p = 0.06). There was also a trend for supplementation to improve teacher-rated oppositional behaviour in 3 studies that supplemented with > 100mg EPA (N = 279, SMD = 0.22; 95% CI: -0.02 to 0.45, z =1.79, p = 0.07). The only evidence of heterogeneity was in those with high EL (and related domains) for teacher rated oppositional behaviour (x^2 = 4.85, I^2 = 79.4%, p = 0.03). Main effects from the sub-group analyses are shown in

Table **3-1**.


Emotional lability (parent rated)
Study
ID
ES (95% CI)
%
Weight
Milte et al (2012) -0.05 (-0.56, 0.46) 13.98
Richardson & Puri (2002) 0.48 (-0.26, 1.22) 7.47
Richardson et al (2012) 0.28 (0.07, 0.49) 41.83
Sinn et al (2007) 0.25 (-0.26, 0.76) 13.99
Manor et al (2012) -0.16 (-0.52, 0.21) 22.72
Overall (I-squared = 29.0%, p = 0.228) 0.15 (-0.07, 0.36) 100.00
-1.5 -1 -.5 0 .5 1 1.5
Favours Placebo
Favours PUFA


Emotional lability (teacher rated)
Study
ID
ES (95% CI)
%
Weight
Richardson & Montgomery (2005) 0.29 (-0.10, 0.68) 25.61
Richardson et al (2012) -0.11 (-0.32, 0.09) 46.84
Manor et al (2012) 0.14 (-0.23, 0.51) 27.55
Overall (I-squared = 47.5%, p = 0.149) 0.06 (-0.19, 0.31) 100.00
-1.5 -1 -.5 0 .5 1 1.5
Favours Placebo
Favours PUFA


Oppositional behaviour (parent rated)
Study
ID
ES (95% CI)
%
Weight
Gustafsson et al (2010) 0.00 (-0.41, 0.41) 11.67
Milte et al (2012) 0.06 (-0.46, 0.58) 7.49
Richardson & Puri (2002) 0.31 (-0.42, 1.04) 3.79
Richardson et al (2012) 0.27 (0.06, 0.47) 38.73
Sinn et al (2007) 0.50 (-0.02, 1.01) 7.48
Stevens et al (2003) -0.11 (-0.78, 0.56) 4.51
Manor et al (2012) -0.03 (-0.39, 0.33) 14.71
Widenhorn-Müller et al (2014) -0.16 (-0.57, 0.25) 11.63
Overall (I-squared = 6.0%, p = 0.384) 0.13 (-0.01, 0.27) 100.00
-1.5 -1 -.5 0 .5 1 1.5
Favours Placebo
Favours PUFA


Oppositional behaviour (teacher rated)
Study
ID
ES (95% CI)
%
Weight
Gustafsson et al (2010) 0.22 (-0.19, 0.63) 11.72
Richardson & Montgomery (2005) 0.28 (-0.11, 0.67) 12.94
Richardson et al (2012) -0.09 (-0.30, 0.11) 46.36
Stevens et al (2003) 0.13 (-0.59, 0.84) 3.84
Manor et al (2012) 0.01 (-0.36, 0.39) 14.30
Widenhorn-Müller et al (2014) 0.13 (-0.30, 0.55) 10.84
Overall (I-squared = 0.0%, p = 0.538) 0.04 (-0.10, 0.18) 100.00
-1.5 -1 -.5 0 .5 1 1.5
Favours Placebo
Favours PUFA

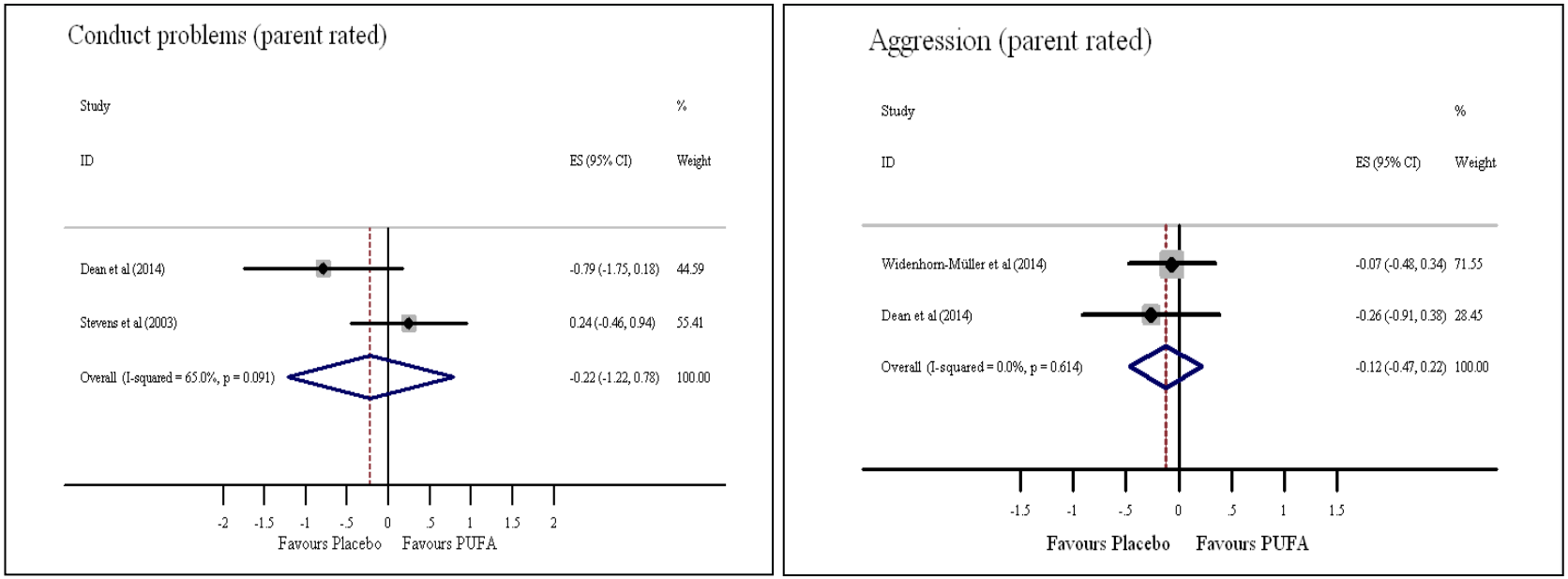


Figure 3-2: Forest plots for meta-analyses across the 6 behavioural domains

ES = Effect Size, ID = Identification

Table 3-1: Subgroup meta-analyses of those studies which: 1) strictly met inclusion criteria, 2) were high quality, 3) supplemented with > 100mg EPA or 4) included those with elevated impairments of emotional lability and related domains.

Sub-analyses **Domain**	**Studies**	**N**	***P***	**SMD[e]**	**95% CI**	**Heterogeneity**	
						P	***I²* (%)**
1. Strict Inclusion[a]							
Emotional lability (parent rated)	[1–4]	515	0.005	0.25	0.08 to 0.43**	0.62	0.0
Emotional lability (teacher rated)	[3,5]	464	0.79	0.05	-0.34 to 0.44	0.07	68.7
Oppositional behaviour (parent rated)	[1–4,6–8]	734	0.06	0.15	-0.006 to 0.31	0.37	8.0
Oppositional behaviour (teacher rated)	[3,5–8]	673	0.55	0.05	-0.11 to 0.20	0.40	1.4
2. High Quality[b]							
Emotional lability (parent rated)	[2,3,9]	526	0.36	0.16	-0.18 to 0.50	0.09	58.2
Emotional lability (teacher rated)	[3,5,9]	598	0.65	0.06	-0.19 to 0.31	0.15	47.5
Oppositional behaviour (parent rated)	[2,3,9]	532	0.02	0.20	0.03 to 0.38*	0.37	0.2
Oppositional behaviour (teacher rated)	[3,5,9]	596	0.86	0.02	-0.19 to 0.23	0.25	28.6
3. Adequate EPA[c]							
Emotional lability (parent rated)	[1,2,4]	153	0.30	0.17	-0.15 to 0.50	0.48	0.0
Oppositional behaviour (parent rated)	[1,2,4,6,8]	337	0.46	0.09	-0.14 to 0.31	0.36	8.3
Oppositional behaviour (teacher rated)	[5,6,8]	279	0.07	0.22	-0.02 to 0.45	0.87	0.0
Aggression	[8,10]	111	0.48	-0.12	-0.47-0.22	0.61	0.0
4. High EL and Related Domains[d]							
Oppositional behaviour (teacher rated)	[6,9]	117	0.20	0.57	-0.30 to 1.43	0.03*	79.4

Note. N = Number of participants

a. Conduct problems (parent rated) and aggression (parent rated) were not included in this as these domains did not include the study which was to be excluded for this analysis (Manor et al., 2012).

b. Conduct problems (parent rated) and aggression did not include any high quality studies so these sub-group analyses could not be run.

c. Emotional lability (teacher rated) and conduct problems (parent rated) included only one study that supplemented with > 100mg EPA so analyses could not be run.

d. Data from the two included studies (Gustafsson et al., 2010; Manor et al., 2012) were only available for teacher rated oppositional behaviour therefore the analyses could be run only in this domain.

e. + tive SMD favours a treatment effect for the *n*-3 PUFA group; - tive SMD favours a treatment effect for the placebo group

* Significant at nominal level (p <.05)

**Significant after correction for multiple testing (p<.008)

Studies

1 = Milte et al., (2012), 2 = Richardson and Puri, (2002), 3 = Richardson et al., (2012), 4 = Sinn and Bryan (2007), 5 = Richardson & Montgomery (2005), 6= Gustafsson et al., *(*2010), 7 = Stevens et al., (2003), 8 = Widenhorn-Müller et al., (2014), 9 = Manor et al., (2012), 10 = Dean et al., (2014).

3.5 Discussion

This systematic review and meta-analysis examines the efficacy of *n*-3 PUFA supplementation on EL, oppositional behaviour, conduct problems, and aggression in children with ADHD and related neurodevelopmental disorders (ADHD+RND). The initial analyses found no significant treatment effects on EL, oppositional behaviour and conduct problems and aggression. However, evidence from the subgroup analyses suggests effects could be present. Significant effects emerged for *n*-3 PUFA supplementation on parent rated EL in studies that met strict inclusion criteria, and parent rated oppositional behaviour in high quality studies (although the latter effect did not withstand correction for multiple testing and is seen as indicative of an effect). There was also a trend for supplementation to improve; teacher-rated oppositional behaviour in studies that supplemented with adequate EPA and parent-rated oppositional behaviour in studies that met strict inclusion criteria. An important observation from this analysis is that despite public interest into omega-3 as

a treatment for ADHD and related disorders, we only identified a small number of studies with sufficient data to be included in the analysis (n=10). This demonstrates the need for further research to be conducted in this area in order to gain a more conclusive picture.

The subgroup analyses suggest the possibility of a small effect (SMDs ranged from 0.15-0.25) of *n*-3 PUFA on EL and oppositional behaviour. The effect, which remained significant after correction for multiple testing (p=.005), was found for *n*-3 PUFA on parent-rated EL, but only after removal of one study that supplemented with phosphatidylserine (PL) containing omega-3 (Manor et al., 2012). Previous research has found superior effects of *n*-3 PUFA + PL (compared to *n*-3 PUFA alone) on ADHD symptoms (Vaisman et al., 2008). Therefore although it is unlikely that adding PL to *n*-3 PUFA would diminish the effect size, given the current finding, it is possible that PL could have the opposite effect on EL that it had on ADHD symptoms by some as yet unknown mechanism. As a whole, the effects and trends found here emerged in higher quality studies that supplement with *n*-3 PUFA only or with higher levels of EPA. This could provide a basis for methodological recommendations for future studies. There was no evidence of heterogeneity suggesting these effects or trends to be consistently spread across the various independent studies.

The suggestion of a possible effect of *n*-3 PUFA on EL and oppositional behaviour is in line with epidemiological and cross-sectional studies which have associated deficiencies in *n*-3 PUFA with conduct problems, temper tantrums and violent behaviour in children, adolescents and adults with and without ADHD (Corrigan et al., 1994; Gow et al., 2013; Hibbeln, 2001; Iribarren et al., 2004; Stevens et al., 1996). The mechanisms behind this link are proposed to be through disruption of neural pathways that regulate behaviour and alterations in serotonin and dopamine levels (Assisi et al., 2006; Haag, 2003; Hibbeln et al., 2006; Young & Conquer, 2005). Alterations in dopamine seem particularly plausible given that abnormalities in this neurotransmitter may underlie ADHD (Bolea-Alamañac et al., 2014) and that EL is a characteristic feature of the disorder (American Psychiatric Association, 2013).

The results here are also in line with a previous meta-analysis in eight studies by Benton, (2007) which found a significant reduction in aggression after supplementation with *n*-3 PUFA (SMD=0.61) in children and adults who were either healthy or had various mental health conditions. This meta-

analysis was however, subject to a number of limitations and differences from the current analysis. Only one of the eight studies from Benton's paper were deemed suitable for the current study (Hirayama et al., 2004) of which we could not obtain data. We combined similar rating scale measures, whereas Benton combined studies which employed psychological tasks (e.g. the picture frustration study) (Hamazaki et al., 2002; Hamazaki et al., 1996) with rating scale measures. We analysed only an ADHD+RND group, whereas Benton combined populations who were typically developing or had various psychiatric diagnoses (e.g. ADHD and borderline personality disorder). One of the papers included in Benton's paper also failed to report results from the placebo group (Fontani et al., 2005). Finally, five of the papers included by Benton were from the same research group (Hamazaki et al., 1998, 2002; Hamazaki et al., 1996; Hirayama et al., 2004; Itomura et al., 2005). Therefore further evidence is required to support our interpretation. Two studies which were included in the qualitative but not quantitative synthesis failed to find treatment effects which may be due to small sample sizes (n=37-40) and heterogeneous study populations. Hirayama et al., (2004) found no effect on parent- and teacher- rated aggression after eight weeks of supplementation in children with ADHD. Bélanger et al., (2009) found no improvement in parent ratings of oppositional behaviour and emotional lability after supplementation for 16 weeks in children with ADHD.

Here we do not find significant effects in our primary analysis, although in exploratory secondary analyses we do find suggestive evidence for small effects of *n*-3 PUFA supplementation on improving EL and oppositional behaviour. One potential limitation leading to non-significant effects is that the majority of studies included in the meta-analysis were underpowered to detect small effects. Effect sizes ranged from 0.15-0.25, in line with previous meta-analyses which found small but significant effects of *n*-3 PUFA on ADHD symptoms in 699 (SMD=0.31, $p < .0001$) (Bloch & Qawasmi, 2011) and 827 (SMD=0.21, p=0.007) (Sonuga-Barke et al., 2010) children with ADHD. With such modest effect sizes around 0.3, we would require a sample size of around 352 participants (β=80%, two tailed α=0.05) and around 548 after correction for multiple testing (β=80%, two tailed α=0.008). In the current study, the included trials ranged from 21 to 362 participants, with only one study in children with reading difficulties being adequately powered (N = 362) (Richardson et al., 2012). Further large-scale studies are therefore needed to confirm or refute such small effects.

Another limitation is that participants within the various studies are heterogeneous with regard to EL and related behavioural domains. This heterogeneity would then account for reduced effect sizes for the outcome measures of interest here, since most of the study samples were selected for high levels of ADHD and RNDs, which show uniform deficits for core ADHD features (Coghill et al., 2007). Similarly, such between subject heterogeneity for secondary outcomes may have affected the results of our recent meta-analysis of *n*-3 PUFA supplementation on cognitive performance deficits, which are found in some but not all children with ADHD and related disorders (Cooper et al., 2015). Such effects might explain why one study found significant treatment effects on aggression for a prison population who had higher and more homogenous symptoms of aggression (Gesch et al., 2002; Zaalberg, Nijman, Bulten, Stroosma, & van der Staak, 2010). We therefore completed one subgroup analyses across two studies which analysed those with more homogenous deficits in emotional lability and oppositional behaviour (Gustafsson et al., 2010; Manor et al., 2012). Although this did lead to a higher estimated effect (SMD=0.57) of *n*-3 PUFA on teacher rated oppositional behaviour, this finding failed to reach statistical significance, yet this could potentially be explained by the very small sample sizes in the individual studies (n=48 and 69).

Another limitation was the substantial between study variation with respect to patient groups, assessment procedures, outcome measures, treatment formulations, and quality in methods adopted for the different studies. This necessitated the use of random effects models that produced wider confidence intervals. Due to reporting deficiencies, the present study used pre-treatment SD instead of SD of the change (the difference before and after the intervention) in the calculation of the effect size (Morris, 2007). This could have resulted in an underestimation of the true effect size (Ortego & Botella, 2010). A sensitivity analysis of two studies of parent rated EL which gave the SD of the change gave a similar, non-significant result, albeit this analyses is limited due to the small number of included studies (see Appendix B, Supplement AB-1).The studies used in this meta-analysis varied in supplement composition and dosage. According to meta-analyses, higher EPA rather than DHA concentrations are associated with symptom reduction in children diagnosed with ADHD (Bloch & Qawasmi, 2011; Puri & Martins, 2014). The current study somewhat supported this, as a trend for supplementation to improve teacher rated oppositional behaviour emerged in studies that supplemented with adequate EPA (>100mg); although meta-regression found no effect

of EPA dose on any of the examined behavioural domains. Therefore further evidence is required to support this claim. There are also inherent problems with blinding in studies which supplement with *n*-3 PUFA due to the fishy flavour of the capsules. Above chance guessing occurred in a number of studies, although this would be expected to inflate rather than reduce any treatment effects (Long & Benton, 2013; Milte et al., 2012; Zaalberg et al., 2010). Identical flavouring of the placebo and active capsules must be used to reduce this limitation. A large number (N=6) of the studies included in the qualitative synthesis used an olive oil placebo. Olive oil contains a high concentration of oleic acid, a precursor of oleamide that has been shown to have psychoactive properties (Richardson, 2006). Stevens et al. (2003) found their olive oil placebo to be 'active' in that a significant increase in the *n*-3 PUFA, ALA, was found following supplementation ($p < .05$). An inert substance such as liquid paraffin oil could be more suitable (Peet & Horrobin, 2002).

Length of supplementation has been proposed as a factor to explain a lack of significant treatment effects. However, we found no relationship between length of supplementation and treatment effects. This is in line with a recent meta-analysis which found no relationship between trial duration and efficacy of *n*-3 PUFA in reducing ADHD symptoms (Bloch & Qawasmi, 2011), providing good evidence that the included trials were of adequate length and that outcomes were unlikely to be influenced by trial duration.

It has also been proposed that treatment effects may occur only in those with low *n*-3 PUFA blood levels at baseline. We recently found, in typically developing children and adults with low *n*-3 PUFA status, *n*-3 PUFA supplementation to improve short-term memory (Cooper et al., 2015). Only one of the 12 studies included in the qualitative synthesis examined effects in those with low PUFA. Stevens et al., (2003) supplemented children with ADHD and low blood *n*-3 PUFA levels (compared to a TD control group) and found a significant treatment effect for conduct problems (a primary outcome), although this was only one of two treatment effects out of 16 outcome measures and was only nominally significant. Other studies have examined the effects of supplementation on cognition or mood in typically developing children who were either malnourished (Osendarp et al., 2007) or had low fish consumption (Kennedy et al., 2009) and found no treatment effects. Future studies should preferentially recruit those who are *n*-3 PUFA deficient to clarify any treatment effects.

In conclusion, although these results do not provide a conclusive picture, they provide suggestive evidence that a small effect of *n*-3 PUFA supplementation on EL and oppositional behaviour might be present. They are however sufficient to exclude moderate to major effects across the samples suggesting that supplementation is unlikely to be an effective general approach to reducing EL, oppositional behaviour, and conduct problems in children with ADHD. Potential effects are very small and therefore of questionable clinical significance, although it remains feasible that there are larger effects in a subgroup. In order to clarify the presence of clinically meaningful effects in clinical subgroups, future studies are required which would ideally employ larger sample sizes (>352 participants), supplement with > 100mg EPA, recruit those who are deficient in *n*-3 PUFA with high levels of emotionally labile behaviour and follow stringent procedures for blinding and other trial procedures.

3.6 Chapter 3: Interim summary

Chapter 3 addressed the final section of the first aim of this thesis (see Section 1.4), to document the effect of *n*-3 PUFA supplementation on emotional lability (EL). We found suggestive evidence of a small effect of *n*-3 PUFA supplementation on reducing EL and oppositional behaviour in children with ADHD or with a related neurodevelopmental disorder (Cooper et al., 2016). This evidence, combined with the results of Chapter 2 (Cooper et al., 2015) and existing evidence (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), provide us with the most up to date estimates of the effect of *n*-3 PUFA supplementation on ADHD symptoms and associated impairments in cognition and emotional lability. There appears to be a small to moderate effect of *n*-3 PUFA supplementation on reducing symptoms of ADHD, no effect (aside from a potential small effect in those who are *n*-3 PUFA deficient) on cognitive performance, and suggestive evidence of a small effect on reducing EL and oppositional behaviour. Results from the reviews in Chapters 2 and 3 showed that there are as yet no published trials of the effect of *n*-3 PUFA supplementation in adults with ADHD. In line with this, the second aim of this thesis was to examine the effect of *n*-3 PUFA supplementation in adults with ADHD (see Section 1.4). The following data chapter (Chapter 5) therefore examined, through the use of a randomised controlled trial (RCT) design with baseline case/control comparisons, the effect of *n*-3 PUFA supplementation on ADHD symptoms, cognitive performance and EL in adults with ADHD. The final data chapter (Chapter 6) examines the effect of

another alternative treatment, the cannabinoid medication Sativex oromucosal spray, in adults with ADHD (the link between this and previous chapters will be discussed in the interim summary of Chapter 5). The following chapter (Chapter 4) discusses the methodologies used in these two randomised controlled trials.

Chapter 4: Methods

4.1 Aims

Data presented in the following two chapters (Chapters 5 and 6) are taken from the OCEAN study (Oils and Cognitive Effects in Adult ADHD Neurodevelopment) and the EMA-C study (Experimental Medicine in ADHD - Cannabinoids). The specific aim of this chapter is to provide a brief summary of the methodologies of these studies. An overview of the outcome measures obtained in these samples is provided in this chapter, however only a number of these measures, relevant to the aims of this thesis, were analysed in Chapters 5 and 6. The remaining data is to be analysed at a later date.

4.2 Part 1: OCEAN Study

4.2.1 Background

4.2.1.1 Location of study, funding and ethical approval

The OCEAN study was conducted at the MRC Social Genetic and Developmental Psychiatry (SGDP) Centre at the Institute of Psychiatry Psychology and Neuroscience (IoPPN) in conjunction with the Adult Attention Deficit Hyperactivity Disorder (ADHD) clinic at the South London and Maudsley Hospital NHS Trust (SLaM). The study was funded by Vifor Pharma (grant number: PADWUDB), awarded to Prof Philip Asherson (Primary Investigator) as an investigator led project (with King's College London as the sponsors). Research ethics approval for this study was granted by the National Research Ethics Service (NRES) Committee London - Camberwell St Giles (reference: 11/LO/1042). Full informed consent was given by all subjects participating in the study.

4.2.1.2 Design

The study was a single-centre 6-month double-blind, placebo controlled, parallel-group pilot study of *n*-3 PUFA supplementation in 81 adults with ADHD. Testing sessions occurred at time 1 (baseline), time 2 (3 months) and time 3 (6 months). A sample of 30 psychiatrically healthy control participants took part in the baseline assessments in order for case/control comparisons to be conducted (see Figure 4-1) (for further details see Chapter 5, Section 5.3.2).

4.2.2 Recruitment

4.2.2.1 Inclusion and exclusion criteria

Eligible participants were adults aged between 18 and 55 years. They had either a formal (a pre-existing diagnosis by a psychiatrist) or research (previously undiagnosed patients (see research diagnosis Section 4.2.2.4) diagnosis of ADHD. Participants met criteria for ADHD with either the combined type presentation or inattentive (with some hyperactive/impulsive symptoms) type presentation. We excluded cases that only presented with inattentive symptoms as there is some evidence this might reflect a separate condition (Barkley, 2014). Age of onset was set at age 12 or below, in accordance with DSM-5 (American Psychiatric Association, 2013). They met all other criteria for DSM-5 ADHD at baseline including pervasive impairments from the symptoms in more than one setting. Participants were either on stable treatment (> 1 month) with ADHD medication (stimulant or non-stimulant treatment such as atomoxetine), or no medication, and could also be taking a low dose of a concomitant medication for depression or anxiety disorders. Participants on stimulant medication must have been willing to come off this medication for 48 hours before the baseline and final (6 month) testing sessions.

Combined type

In childhood: ≥ 6 symptoms of inattention; and ≥ 3 symptoms of hyperactivity-impulsivity.
In adulthood: ≥ 5 symptoms of inattention; and ≥ 3 symptoms of hyperactivity-impulsivity.

Inattentive type

In childhood: ≥ 6 symptoms of inattention; and ≥ 3 symptoms of hyperactivity-impulsivity.
In adulthood: ≥ 5 symptoms of inattention; and ≥ 2 symptoms of hyperactivity-impulsivity.

Participants were excluded who had a current or historical diagnosis of autism spectrum disorder (ASD), tourette's syndrome, bipolar I disorder, any psychotic disorder, obsessive compulsive disorder (OCD), frequent panic attacks, or general learning difficulties (defined as a IQ < 80). As a lifetime history of depression is so common amongst this population, only those with recurrent depression or those in a current depressive episode at the time of contact were excluded. Neurological problems, head injury, current or previous substance abuse or frequent substance use (more than 8 units (6 units for females) of alcohol consumed daily or recreational drug use more

than twice weekly) were also excluded; as were participants with any major physical health problems (including diabetes, thyroid problems, cancer, or an infectious disease). Participants with fish allergies or who had taken omega-3 or 6 supplements in the previous 3 months were excluded.

4.2.2.2 Recruitment sources

Controls

Controls were recruited via recruitment circulars and advertisements in the local community. Eligible controls were age, gender and IQ matched to the patient sample and fulfilled all the same exclusion criteria. Additionally, control participants screened below threshold for ADHD on the Adult Self Rating Scale for ADHD (ASRS) (Appendix C, Table AC-1) (Kessler, Adler, Ames, et al., 2005).

Cases

Recruitment occurred through four sources:

1. Recruitment through South London and Maudsley NHS Trust (SLaM): The medical records of patients (either follow-up patients or those on the waiting list) from the SLaM Adult ADHD Service were screened for eligibility, using the inclusion and exclusion criteria, by a member of the OCEAN research team (myself, CS or RS) who held honorary clinical contracts. In addition previous ADHD study databases were also screened for suitable participants. Those deemed eligible were sent study information sheets, invitations, a response slip and a stamped addressed envelope. Where no response slip was returned, participants were contacted by telephone to determine their interest in participating. Those who expressed an interest in participating in the study completed a telephone screening (detailed in Section 4.2.2.4). If deemed suitable following the telephone screening and if a Conners Adult ADHD Diagnostic Interview for DSM-IV (CAADID: a structured clinical interview for the 18 ADHD symptoms in childhood and adulthood) (Epstein, Johnson, & Conners, 2001) had been completed as part of their diagnostic assessment at SLaM, then they were invited into the trial and their baseline assessment was booked. If a CAADID were not completed as part of their diagnostic assessment it was completed over the phone by a member of the research team (RS or CS). If the patient was on the waiting list then a research diagnostic assessment was carried out by Prof Asherson and myself (detailed in Section 4.2.2.4).

2. Online questionnaire: In order to recruit undiagnosed patients an online screening questionnaire was set-up (http://neuroknowhow.com/adhdoraddquestionnairepage/) (although this link has now been disabled). This was established by a study participant who runs the website 'neuroknowhow' (http://neuroknowhow.com/aboutus/) which provides services and online help for those with neurodevelopmental difficulties such as ADHD, dyslexia, and dyspraxia. The screening questionnaire consisted of the six questions in Part A of the(ASRS) which have been found to be the most predictive of ADHD (Kessler, Adler, Ames, et al., 2005) (Appendix C, Table AC-2). Those who screened above the threshold for ADHD were asked to complete the Barkley Childhood Behaviour Scale (Appendix C, Table AC-3). If they scored positive for 6 or more symptoms of either or both domains of inattention or hyperactivity/impulsivity then a research assessment was conducted (see Section 4.2.2.4).

3. Online advertisements: Participants were also recruited from advertisements on the ADHD support websites AADD-UK (Adult ADHD-UK) (http://aadduk.org/about/) and ADDISS (The National Attention Deficit Disorder Information and Support Service) (http://www.addiss.co.uk/). We were also contacted from participants who saw the trial registered on clinical trials.gov (identifier: NCT01750307). If these participants had an existing diagnosis we asked them to send us a copy of their diagnostic assessment report. If inclusion/exclusion criteria were met then the CAADID (Epstein et al., 2001) was completed by RS or CS. If the participants did not have an existing diagnosis and screened above threshold for ADHD on the ASRS (Kessler, Adler, Ames, et al., 2005) and Barkley's Childhood Behaviour Scales (Barkley, 1998) then a research assessment was conducted (see Section 4.2.2.4).

4. Recruitment through other doctors: We attended the clinical team meetings at the Maudsley Adult ADHD Clinic to communicate the study to members of the healthcare team and ask if they had any suitable patients and if they could let their patients know about the study. The study was also circulated to clinicians on the email list of the UK Adult ADHD Network (UKAAN). Figure 4-1 details the recruitment path.

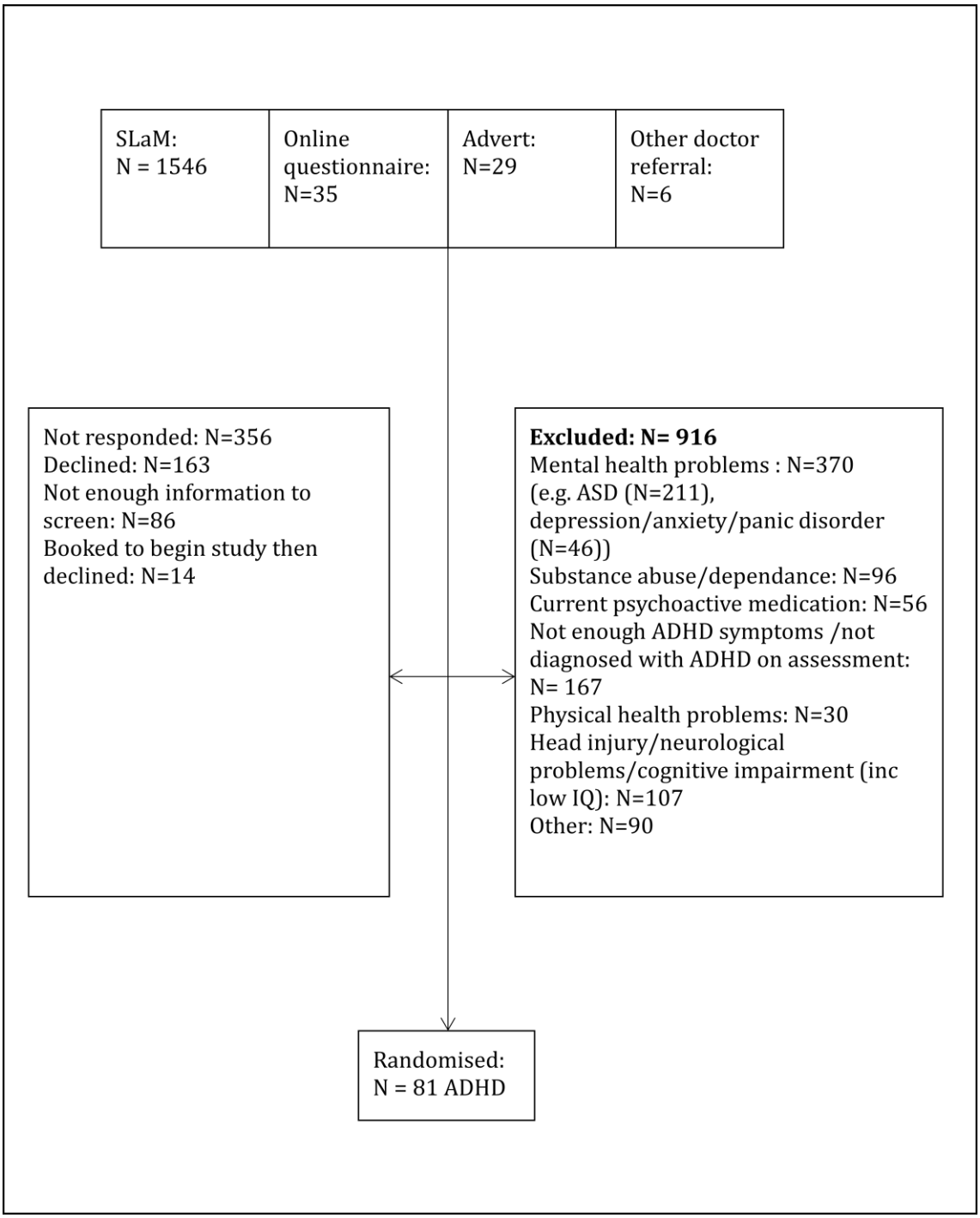


Figure 4-1: Flow diagram of recruitment and exclusions for the OCEAN study (see Appendix C, Table AC-4 for a more detailed breakdown of exclusions).

Note. ASD = Autism Spectrum Disorder, Participants who overlapped mental health/substance abuse exclusion categories: N=89.

4.2.2.3 Telephone screening

Both ADHD and control participants underwent a structured telephone screening of exclusionary criteria, which consisted of detailed questions assessing any previous or current mental health problems including: presence, treatment for or diagnosis of anxious, depressive and manic/hypomanic symptoms, physical health problems, neurological problems, drinking and drug habits, use of omega-3 or 6 supplements, and any known allergies to fish (See Appendix C, Supplement AC-1).

4.2.2.4 Research assessment

Undiagnosed participants who met inclusion/exclusion criteria were asked to complete (over the telephone with myself) the CAADID (Epstein et al., 2001). In line with DSM-5, symptom onset and chronicity was established before age 12 and in adulthood, the presence of a minimum of 5 symptoms of inattention and 3 symptoms of hyperactivity/impulsivity were established (American Psychiatric Association, 2013). The CAADID was also completed (over the telephone by myself) with someone who knew the participant in childhood, most commonly a parent. The CAADID was then reviewed by Prof Philip Asherson, an experienced consultant psychiatrist specialising in adult ADHD, who approved the participants prior to inviting them into the study. In addition, Prof Asherson met participants at their baseline assessment to review and confirm the diagnosis. Participants were then provided with a letter from Prof Asherson detailing the outcome of the research assessment. Participants who had not yet been referred or diagnosed for adult ADHD could then, if they wished, use this letter to help gain a referral for a formal adult ADHD assessment, although they were asked to not begin medication for the duration of the trial if they wished to take part.

4.2.2.5 Exclusions

The recruitment path and reasons for exclusions are shown in Figure 4-1 and in detail in Appendix C, Table AC-4. Of the 1616 individuals screened for the study 916 were excluded. The main reason for exclusion was due to co-occurring mental health conditions (40.4% of the total excluded sample). The most common being either diagnosed with or having suspected ASD (23%),

anxiety/panic disorder and depression (5.0%), OCD (3.7%), psychosis/schizophrenia (2.0%), bipolar disorder (2.5%) and those with complex comorbid conditions (1.9%). The next most common reason for exclusion was due to the individual not being diagnosed with ADHD on assessment or having too few symptoms (18.2%), head injuries, neurological problems or cognitive impairment (11.7%), substance abuse or dependence (10.5%) (most commonly cannabis (5.7%) use), and use of psychoactive medications (6.1%) were other common reasons for exclusion.

4.2.2.6 Alteration to exclusion criteria

Due to the high levels of comorbidity in adults with ADHD, mid-way through the study the exclusion criteria were altered, so that patients taking antidepressant medication were included, so long as they met research diagnostic criteria for ADHD and all other inclusion and exclusion criteria. The final sample for the OCEAN study was therefore a relatively representative sample including some (N=14) cases with comorbid difficulties with mild depression and anxiety without panic symptoms, and the sample was also mixed sex. There was no difference in ADHD symptom severity between those who were taking concomitant antidepressant medication and the remaining sample (who were either unmedicated or medicated with stimulant or non-stimulant medication) ($p > .05$) (see Appendix C, Table AC-5).

4.2.3 Participants

The participants were 81 adults with ADHD (mean age = 33.5 years (10.26)) and 30 typically developing control participants, matched roughly in age ($p=0.06$) and IQ ($p=0.41$), although there was a trend for the controls to be slightly younger than the ADHD cases (for further details see Chapter 5, Section Error! Reference source not found.).

4.2.4 Research assessment tools

The following is an overview of the measures used in the three testing sessions (Baseline, Time2 and Time 3). Table 4-1 summarises which assessments were made at each time point. A detailed description of the data used in the analyses is presented in Chapter 5 (Section 5.3.6).

Baseline only measures

- **The MINI 6.0 (Mini International Neuropsychiatric Interview) diagnostic interview** (Lecrubier et al., 1997)**:** The MINI was used to screen for comorbid disorders.
- **The Weschler Abbreviated Scale of Intelligence – II (WASI-II)** (Weschler, 2005): Two subtests (vocabulary and matrix reasoning) of the WASI-II were used to measure IQ.
- **Socio-economic status (SES):** SES was assessed by collecting information on participants' level of education, occupation and income.
- Information regarding the participants' medication and when they were diagnosed with ADHD was also collected.

Assessment of efficacy

Primary efficacy measure

Sustained Attention to Response Task (SART) (O'Connell et al., 2009)**:** The SART is a computerised go/no go task. The primary outcome was performance measured through number of commission errors (where the participant responds where a response is not required) (see Chapter 5, Section 5.3.6.1 for further details).

Secondary efficacy measures

ADHD symptoms:

- ***Conners' Adult ADHD Rating Scales (CAARS)*** (Conners, Erhardt, & Sparrow, 1999) ***and Wender-Reimherr Adult Attention Deficit Disorder Scale (WRAADS)*** (Wender, 1995) ***combined (investigator rated):*** Assessed investigator rated ADHD symptom severity.

Self-report questionnaires:

- ***Executive function:*** Behavioural Rating Inventory of executive function – Adult Version (Brief-A) (Roth, Isquith, & Gioia, 2005).
- ***Common psychopathology:*** Symptom Checklist-90 (*SCL*-90) (Derogatis & Unger, 2010).
- ***Sleep:*** The Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, & Monk, 1989).
- ***Thoughts:*** The Depressive Thoughts Questionnaire (DTQ) (Clark & De Silva, 1985) assesses levels of depressive thoughts; and the Cognitive Control Questionnaire (CCQ) (Asherson, unpublished report) assesses the control they have over their thoughts, a concept similar to excessive mind wandering.

- ***Life Stress:*** The Brief COPE questionnaire (Carver, 1997) assesses how they are coping with stressful life events and the Brief Life Events Questionnaire (BLEQ) (Brugha & Cragg, 1990) assesses the occurrence of stressful life events over four months.
- ***Functional Impairment Questionnaires:*** The Weiss Functional Impairment Rating Scale Self Report (WFIRS-S) (Weiss, 2007) and the Adult ADHD Quality of Life Scales (AAQoL) (Brod, Johnston, Able, & Swindle, 2006) cover various aspects of social and cognitive function, productivity, health and relationships.
- ***Reading:*** The word reading and spelling subtests of the Wechsler Individual Achievement Test (WIAT-II) (Wechsler, 2005) were used to assess reading and spelling ability.
- ***Fish frequency questionnaire (FFQ):*** This is a brief questionnaire which asks participants to rate their average fish intake for the prior 6-months.

Cognitive function measured by electroencephalography

- An electroencephalography (EEG) recording session lasting approximately 1.5 hours was carried out. Conditions or tasks implemented during the EEG recording are outlined below and were administered in the order with which they are described. All tasks were administered using the presentation software package (www.neurobs.com). A brief description of each task is provided below.

  Two resting state conditions (first eyes open, then eyes closed), lasting 3 minutes each were carried out at the beginning and end of each recording session. Measures of sustained attention, inhibition, reaction time, and reaction time variability were obtained using the Sustained Attention to Response Test (SART) (task duration was 15 minutes) (O'Connell et al., 2009), and the Cued Continuous Performance Test (CPT-OX) with flankers (McLoughlin et al., 2010; Valko et al., 2009) (task duration was 11 minutes). Measures of reaction time and reaction time variability were obtained using the Fast Task (Andreou et al., 2007; Kuntsi et al., 2006) (task duration was 18 minutes). A more detailed description of these tasks is available in Chapter 5.

Emotional lability

- ***Mood self-report questionnaires:*** The Centre for Neurologic Study Lability Scale (CNS-LS) (Moore, Gresham, Bromberg, Kasarkis, & Smith, 1997) and Affective Lability Scale-Short Form (ALS-SF) (Oliver & Simons, 2004) measured emotional lability.
- ***The Computerized Paced Auditory Serial Addition Task*** *(PASAT-C* (Lejuez, 2003)*)*: This is a computerised mental arithmetic task used to elicit frustration (a more detailed description of this task is available in Chapter 5).

4.2.5 Blood samples

In order to monitor compliance 2 x 10ml blood were collected from each of the ADHD cases at all three time points. In order for case/control comparisons to be made 2 x 10ml blood were collected from each control at the baseline assessment. Omega-3 and 6 blood levels were measured.

4.2.6 Randomisation

Randomisation was carried out (by Vifor Pharma) (see Chapter 5, Section 5.3.9 for further details).

4.2.7 Supplementation

Participants in the active group were supplemented with Equazen High concentrated (Equazen HC). Participants in the placebo group were supplemented with medium chain triglycerides (an inert fatty acid).

4.2.8 Testing procedure

The testing procedure is detailed in Chapter 5, Section 5.3.7. Table 4-1 details the assessments and timings at each time point.

Table 4-1: OCEAN assessments and timing

Measure	Time to complete	Time 1 Baseline SGDP	Time 2 3-months SGDP	Time 3 6-months SGDP
EEG	1.5-hours	X		X
WIAT-II	30 minutes	X		X
IQ (WASI)	20 minutes	X		
PASAT	20 minutes	X		X
MINI	30 minutes	X		
SES + Medication info	10 minutes	X		
CAARS/WRAADDS	10 minutes	X	X	X
Blood sample	10 minutes	X	X	X
Fish frequency questionnaire*	5 minutes	X	X	X
BRIEF-A*	5 minutes	X	X	X
SCL-90*	5 minutes	X	X	X
CNS-LS*	5 minutes	X	X	X
ALS-SF*	5 minutes	X	X	X
WFIRS-S*	5 minutes	X	X	X
PSQI*	5 minutes	X	X	X
DTQ*	5 minutes	X	X	X
CCQ*	5 minutes	X	X	X
Brief-cope*	5 minutes	X	X	X
BLEQ*	5 minutes	X	X	X
AAQoL*	5 minutes	X	X	X
Total time		4 h 40m	1h 20m	3h 40m

Note. * = Completed at home, EEG = electroencephalography, WIAT-II = Weschler Individual Achievement Test (2nd edition), IQ = Intelligence quotient, WASI = Weschler Abbreviated Scale of Intelligence, PASAT = Paced Auditory Serial Addition Task, MINI = Mini International Neuropsychiatric Interview, SES = Socio-Economic Status, CAARS = Conners' Adult ADHD Rating Scales, WRAADDS = Wender-Reimher Adult Attention Deficit Disorder Scale, BRIEF-A = Behavior Rating Inventory of *Executive Function* –Adult Version, SCL-90 = Symptom Checklist-90, CNS-LS = Centre for Neurologic Study Lability Scale, ALS-SF = Affective Lability Scale-Short Form, WFIRS = Weiss Functional Impairment Rating Scale Self Report, PSQI = The Pittsburgh Sleep Quality Index, DTQ = Depressive Thoughts Questionnaire, CCQ = Cognitive Control Questionnaire, BLEQ = Brief Life Events Questionnaire, AAQoL = Adult ADHD Quality of Life Scales. SGDP = Testing sessions occurred at the Social, Genetic and Developmental Psychiatry Centre.

4.2.9 Safety

Safety measures during the study involved:

- A full clinical history taken at the time of recruitment, including both medical and psychiatric disorders (and new symptoms arising during the course of the study).
- History of allergy to fish oil products (an exclusion from the study).
- Vital Signs (pulse, blood pressure) and body weight were measured at all study visits.

4.2.10 Preparatory work

4.2.10.1 Power

See Chapter 5, Section 0.

4.2.10.2 Piloting

Before testing began, the project was piloted on eight adult participants recruited from the University campus.

4.3 Part 2 EMA-C Study

4.3.1 Background

4.3.1.1 Location of study, funding and ethical approval

This was a single-centre trial conducted between November 2014 and July 2015 at the SGDP Centre, London in conjunction with the Adult Attention Deficit Hyperactivity Disorder (ADHD) clinic at SLaM. The study was funded by a department research account for PA, with the active and placebo medication provided free of charge by GW Pharma. Research ethics approval for the study was granted by the National Research Ethics Service (NRES) Committee London – London Bridge (reference: 14/LO/0606). The study was conducted in compliance with the principles of the Declaration of Helsinki (Amendment 7), the principles of GCP and all applicable regulatory requirements.

4.3.1.2 Design

The study was a 6 week double blind, placebo controlled, parallel group pilot study of Sativex oromucosal spray in 30 adults with ADHD (for further details see Chapter 6, Section 6.4.1) Testing sessions occurred at baseline and 6 weeks.

4.3.2 Recruitment

4.3.2.1 Inclusion and exclusion criteria

The study was open to males and females aged 18-55 who met DSM-5 criteria for ADHD (N=30). Participants had either a formal (pre-existing diagnosis by a psychiatrist) or research (previously undiagnosed patients) diagnosis of ADHD. In both cases the diagnosis was checked by PA to ensure they met DSM-5 criteria (see research diagnosis Section 4.3.2.4). Participants were required to have the combined type presentation of ADHD and we excluded those with only inattentive symptoms. The following criteria were applied:

In childhood: ≥ 6 symptoms of inattention; and ≥ 3 symptoms of hyperactivity/impulsivity.
In adulthood: ≥ 5 symptoms of inattention; and ≥ 3 symptoms of hyperactivity/impulsivity.

Age of onset was set at 12 or below, in accordance with DSM-5 criteria (American Psychiatric Association, 2013). All cases met other criteria for ADHD at the time of the baseline assessment (i.e.

pervasive impairment from the symptoms in more than one setting). Participants were required to score > 24 on the 18-item Conners' Adult ADHD Rating Scale (CAARS) (Conners et al., 1999). Participants were either unmedicated or medicated with stimulant medication only and were willing to come off this medication for 1 week before and for the duration of the study. To ensure this did not disadvantage patients, we carefully telephone screened participants in order to include those who did not take their stimulant medication on a regular basis, and where short periods without medication were not thought by both the patient and psychiatrist to represent a clinical problem in the overall control of the symptoms and impairments. Participants were also asked to not use any other prescription or non-prescription medication or recreational drugs during the study.

Exclusion criteria were as follows: a current and primary diagnosis of ASD; recurrent major depression, panic/anxiety disorder; bipolar I disorder; any psychotic disorder; OCD; tourette's or general learning difficulties defined as an IQ < 70; Neurological problems; a known or suspected history of drug or alcohol dependence; a first degree relative with a psychotic disorder or use of non-stimulant ADHD medication; those who were using or had used cannabis or cannabis based medications in the 30 day period prior to study entry; concurrent history of renal, hepatic, cardiovascular or convulsive disorders; females who were pregnant or breastfeeding or female participants of child bearing potential, and male subjects whose partner was of child bearing potential, who were unwilling to ensure that they or their partner used two effective forms of contraception (for example, oral contraception, double barrier, intra-uterine device) during the study and for three months thereafter (this is because there is not enough information to say that Sativex is safe in pregnancy).

4.3.2.2 Recruitment sources

There were three main sources of recruitment for the ADHD cases:

1. Recruitment through SLaM or 2. Recruitment from previous research studies: The medical records of patients (either follow-up patients or those on the waiting list) from the SLaM Adult ADHD Service were screened for eligibility (initial screening) using the above inclusion and exclusion criteria by a research assistant (EW) who held an honorary clinical contract. Those

deemed eligible were sent study information sheets, invitations, a response slip and a stamped addressed envelope. Suitable participants from the OCEAN study and two other studies which recruited adults with ADHD (Female Experiences and Brain Activity (FEBA) and Mood Instability Research in ADHD (MIRIAD)) also underwent an initial screening and were either posted letters or emailed. Where no response slip or email reply was received, participants were contacted by telephone to determine their interest in participating. Those who expressed an interest in participating in the study completed a telephone screening (detailed below). If deemed suitable following the telephone screening, and a Diagnostic Interview for ADHD in Adults DIVA (Kooij & Francken, 2010) had been completed as part of their diagnostic assessment at SLaM or as part of the research study then they were invited into the trial and their baseline assessment was booked. If a DIVA was not completed it was completed over the phone by EW. If the patient was on the waiting list and therefore undiagnosed then a research assessment was carried out by EW, and in a face to face interview with Prof Asherson (detailed in Section 4.3.2.4).

3. Online advertisements, 4. Recruitment through other doctors: Participants were recruited from advertisements on the ADHD support websites AADD-UK (http://aadduk.org/about/) and ADDISS (http://www.addiss.co.uk/). We were also contacted from participants who saw the trial registered on clinical trials.gov (identifier: NCT02249299). The study was also communicated to clinicians who were part of the UK Adult ADHD Network (UKAAN). Interested participants with an existing diagnosis were asked to send a copy of their diagnostic assessment report. An initial screening was carried out and if inclusion/exclusion criteria were met, then a telephone screening (Section 4.3.2.3) and then the DIVA were completed by EW. If the participants did not have an existing diagnosis then a research assessment was conducted including a face to face diagnostic assessment with Prof Asherson (see Section 4.3.2.4). Figure 4-2 details the recruitment path.

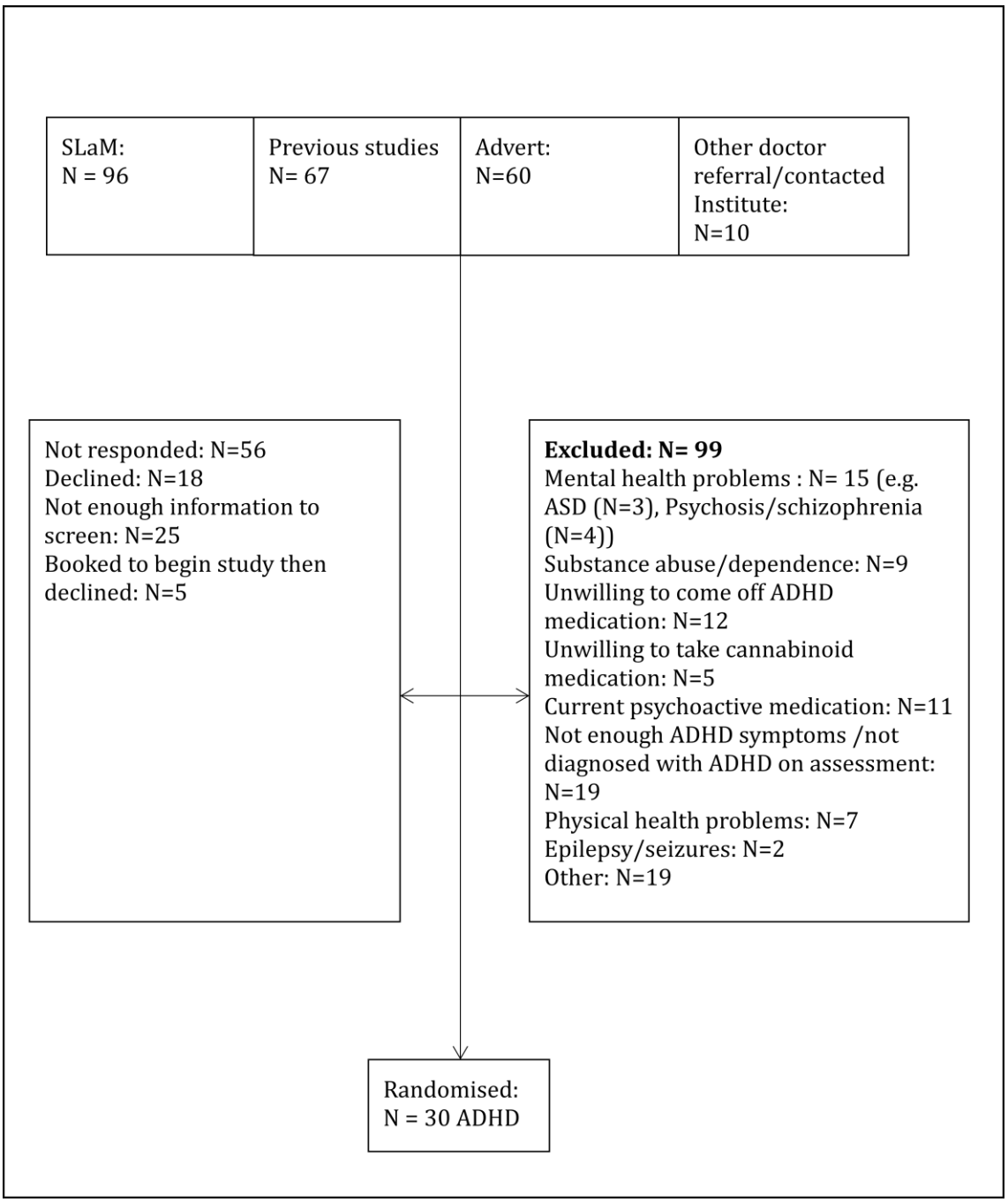


Figure 4-2: Flow diagram of recruitment and exclusions for the EMA-C study (see Appendix C, Table AC-6 for a more detailed breakdown of exclusions).

Note. ASD = Autism Spectrum Disorder, Participants with overlapping mental health/substance abuse exclusion categories: N= 13.

4.3.2.3 Telephone screening

ADHD participants underwent a structured telephone screening for exclusionary criteria, which consisted of detailed questions assessing any previous or current mental health problems (including presence, treatment for or diagnosis of anxious, depressive and manic/hypomanic symptoms), physical health problems, neurological problems, drinking and drug habits. Participants were also asked about their use of ADHD medication. It was also reiterated to participants that there was a 50% chance of receiving the placebo and then asked whether it would be a problem for them to not be on any ADHD medication for the 7 week study (1 week before and the 6 week trial). Participants were also asked about their cannabis use and any previous problems following cannabis use. Finally they completed the 18-item CAARS (Conners et al., 1999) (see Appendix C, Supplement AC-2).

4.3.2.4 Research assessment

Undiagnosed participants who met other inclusion/exclusion criteria were asked to complete (over the telephone with a research assistant (EW[1])) the DIVA (Kooij, 2012). The DIVA is a structured clinical interview for the 18 ADHD symptoms in childhood and adulthood. Symptom onset and chronicity were established before age 12 and the presence of more than 5 symptoms of inattention and 3 of hyperactivity/impulsivity were established in adulthood (in accordance with recent DSM-5 criteria (American Psychiatric Association, 2013). Prof Philip Asherson then spoke to the participants over the phone and met with them at their baseline assessment in order to confirm the diagnosis and approve the participants to enter the study. At the end of the trial these patients were offered treatment for ADHD when this was appropriate. They were registered with SLaM and treated for their ADHD, generally with stimulant medication, by Prof Asherson or another member of the clinical team.

4.3.2.5 Exclusions

The recruitment path and reasons for exclusions are shown in Figure 4-2 and in detail in Appendix C, Table AC-6. Of the 233 individuals screened for the study 99 were excluded. The main reasons for exclusions were that the participant did not have ADHD on assessment or did not have enough

[1]EW was trained in conducting the DIVA by Prof Asherson (a consultant psychiatrist specialising in ADHD)

symptoms (19.2%), or the presence of a co-occurring mental health condition (15.2%), commonly psychosis or schizophrenia (4%), ASD (3%) or bipolar disorder (3%). Other reasons for exclusion included participants who were unwilling to come off their ADHD medication (12.1%), substance abuse or dependence (9.1%), the current use of psychoactive medication (11.1%), or that they were unwilling to take a cannabis-based medication (5.1%).

4.3.3 Participants

The participants were 30 adults with ADHD (11 female, 19 male, mean age = 37.9 years (SD = 11.46) (further details are given in Chapter 6, Section 6.5.1).

4.3.4 Research assessment tools

The following is an overview of the measures used in the two testing sessions (baseline and 6 weeks). Table 4-1 summarises which assessments were made at each time point. A detailed description of the data used in the analyses is presented in Chapter 6, Section 6.4.6).

Baseline only measures

- **The MINI 6.0 (Mini International Neuropsychiatric Interview) diagnostic interview** (Lecrubier et al., 1997): The MINI was used to screen for comorbid disorders.
- **The Weschler Abbreviated Scale of Intelligence – II (WASI-II**) (Weschler, 2005): Two subtests (vocabulary and matrix reasoning) of the WASI-II were used to measure IQ.
- **Socio-economic status (SES)**: SES was assessed by collecting information on participants' level of education, occupation, and income.
- Information regarding the participants' medication, when they were diagnosed with ADHD and history of cannabis use was also collected.

Assessment of efficacy

Primary efficacy measure

QbTest (Quantitative Behaviour Test) (Bijlenga, Jasperse, Gehlhaar, & Kooij, 2015; Iberstadt, 2012)**:** The Qb Test is a 20 minute, unconditional identical pairs test (see Chapter 6, Section 6.4.6.2 for further details).

Secondary efficacy measures

ADHD symptoms:

- ***Conners' Adult ADHD Rating Scales (CAARS)*** (Conners et al., 1999) ***and Wender-Reimher Adult Attention Deficit Disorder Scale (WRAADS)*** (Wender, 1995) ***combined (investigator rated):*** Assessed ADHD symptom severity.

Self-report questionnaires:

- ***Executive function:*** Behavioural Rating Inventory of executive function – Adult Version (Brief-A) (Roth et al., 2005).
- ***Common psychopathology:*** Symptom Checklist-90 (*SCL*-90) (Derogatis & Unger, 2010).
- ***Sleep:*** The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989).
- ***Thoughts:*** *T*he Depressive Thoughts Questionnaire (DTQ) (Clark & De Silva, 1985) assesses levels of depressive thoughts and the Cognitive Control Questionnaire (CCQ) (Asherson, unpublished report) assesses the control they have over their thoughts.
- ***Life Stress:*** The Brief COPE questionnaire (Carver, 1997) assesses how they are coping with stressful life events and the Brief Life Events Questionnaire (BLEQ) (Brugha & Cragg, 1990) assesses the occurrence of stressful life events over four months.
- ***Functional Impairment Questionnaires:*** The Weiss Functional Impairment Rating Scale Self Report (WFIRS-S) (Weiss, 2007) and the Adult ADHD Quality of Life Scales (AAQoL) (Brod et al., 2006) cover various aspects of social and cognitive function, productivity, health and relationships.
- ***Emotional lability:*** The Centre for Neurologic Study Lability Scale (CNS-LS) (Moore et al., 1997) and Affective Lability Scale-Short Form (ALS-SF) (Oliver & Simons, 2004) measured emotional lability.

Cognition

Sustained Attention to Response Task (SART) (O'Connell et al., 2009)**:** The SART is a computerised go/no go task (See Chapter 6, Section 6.4.6.3 for further details)**.**

4.3.5 Randomisation

The randomisation list was produced by an independent statistician using a random number generator in the R statistical package (for further details see Chapter 6, Section 6.4.9).

4.3.6 Treatment

Active treatment

Sativex Oromucosal Spray (GW Pharma Ltd, Salisbury. UK). Each 100 microlitre spray contained: 2.7 mg delta-9-tetrahydrocannabinol (Δ9-THC) and 2.5 mg cannabidiol (CBD).

Placebo treatment

The placebo treatment was an oromucosal spray, containing ethanol, propylene glycol (50:50) excipients, with peppermint oil (0.05%) flavouring and colourings.

For further details see Chapter 6, Section 6.4.5.

4.3.7 Testing procedure

See Chapter 6, Section 6.4.7, Table 4-2 details the assessments and timings.

Table 4-2: Assessments and timings for the EMA-C study

Measure	Time to complete	Baseline (SGDP)	6 weeks (SGDP)
Consent	5 minutes	X	
Information/ demographics	10 minutes	X	X
WASI	30 minutes	X	
MINI 6.0 diagnostic interview	30 minutes	X	
CAARS/WRADDS	10 minutes	X	X
BRIEF-A	5 minutes	X	X
SCL-90	5 minutes	X	X
CNS-LS	5 minutes	X	X
ALS-SF	5 minutes	X	X
WFIRS	5 minutes	X	X
PSQI	5 minutes	X	X
DTQ	5 minutes	X	X
CCQ	5 minutes	X	X
Brief-cope	5 minutes	X	X
BLEQ	5 minutes	X	X
AAQoL	5 minutes	X	X
SART	20 minutes	X	X
QB test	30 minutes	X	X
Total time		3 hours 10 minutes	2 hours 05 minutes

Note. SGDP = Testing sessions occurred at the Social, Genetic and Developmental Psychiatry Centre

Note. WASI = Weschler Abbreviated Scale of Intelligence, MINI = Mini International Neuropsychiatric Interview, CAARS = Conners' Adult ADHD Rating Scales, WRAADDS = Wender-Reimher Adult Attention Deficit Disorder Scale SES = Socio-Economic Status, BRIEF-A = Behavior Rating Inventory of Executive Function –Adult Version, SCL-90 = Symptom Checklist-90, CNS-LS = Centre for Neurologic Study Lability Scale, ALS-SF = Affective Lability Scale-Short Form, WFIRS = Weiss Functional Impairment Rating Scale Self Report, PSQI = The Pittsburgh Sleep Quality Index, DTQ = Depressive Thoughts Questionnaire, CCQ = Cognitive Control Questionnaire, BLEQ = Brief Life Events Questionnaire, AAQoL = Adult ADHD Quality of Life Scales. SART = Sustained Attention

to Response Task, Qb Test = Quantitative Behaviour Test, SGDP = Testing sessions occurred at the Social, Genetic and Developmental Psychiatry Centre.

4.3.8 Safety

Safety measures during the study involved:

- A full clinical history taken at the time of recruitment, including both medical and psychiatric disorders (and new symptoms arising during the course of the study).
- History of cannabis use with no adverse effects.
- Vital Signs (pulse, blood pressure) measured at each study visit. Safety monitoring during dose titration (day 1-14) and days 14-42 (see Section 4.3.8).
- Participants were provided with a 'study card' which stated they were taking part in a clinical trial with a cannabinoid medication. The card contained contact details and two 24 hour emergency phone numbers. The emergency phones were held by myself and Prof Asherson.

4.3.9 Adverse events

Serious adverse events were reported to and investigated by Prof Asherson. Adverse events included any untoward medical occurrence, including any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition. Serious adverse events were recorded and reported by Prof Asherson in line with NRES (National Research Ethics Service) specifications for non-CTIMPs (non-Clinical Trials of an Investigational Medical Product): A safety report form was obtained and completed (from: http://www.hra.nhs.uk/resources/during-and-after-your-study/progress-and-safety-reporting/) and was sent to the study sponsor (King's College London), GW Pharma and the National Research Ethics Service (NRES) Committee London – London Bridge. If necessary Prof Asherson would communicate with the participant's general practitioner or other medical healthcare professional.

4.3.10 Preparatory work

4.3.10.1 Power

See Chapter 6, Section 0.

4.3.10.2 Piloting

Before testing commenced the study assessments were piloted on four volunteers who were recruited from the University campus.

Chapter 5: The OCEAN Study: A Randomised Controlled Trial of Omega-3 Supplementation in Adults with ADHD

5.1 Abstract

Stimulants, atomoxetine and other ADHD medications can be difficult to tolerate or only partially effective or ineffective in some cases. Research into alternative or adjunctive treatments for ADHD is therefore important. One of the most studied alternative treatments in ADHD is omega-3 polyunsaturated fatty acid (*n*-3 PUFA) supplementation. Deficiencies in *n*-3 PUFA blood levels have been commonly found in children and adults with ADHD. Subsequent meta-analyses of *n*-3 PUFA supplementation in children with ADHD estimate small to moderate effects on reducing ADHD symptoms, and provide suggestive evidence for reductions in associated symptoms of emotional lability (EL) and weak evidence for effects on cognition. The effect of *n*-3 PUFA supplementation in adults with ADHD has yet to be established. Given this, we present results from a randomised placebo-controlled trial (RCT) of *n*-3 PUFA supplementation in 81 adults with ADHD. The study also included baseline case/control comparisons which showed that the ADHD cases had significantly impaired cognitive performance (increased omission and commission errors, RTV[2], MRT[3] and CV[4]) and more severe symptoms of ADHD and EL compared to healthy controls. However no case/control difference in *n*-3 PUFA blood levels were found. Results from the primary intent-to-treat analysis for the RCT found supplementation did not improve cognition, EL, or symptoms of ADHD. Using a per protocol analysis, marginal evidence for improvement was found for inattention, and to a lesser extent EL, in those who strictly adhered to the protocol. In conclusion, despite the ADHD group showing increased symptoms and impairment compared to the control group, no evidence for *n*-3 PUFA deficiency was found. Results from the RCT are interpreted in light of limitations due to a high drop-out rate, as providing limited evidence for an effect of *n*-3 PUFA on inattention and potentially symptoms of EL in adults with ADHD.

[2] Reaction time variability.
[3] Mean reaction time.
[4] Coefficient of variation.

5.2 Introduction

Attention-deficit/hyperactivity disorder (ADHD) is characterised by the core behavioural symptoms of impulsivity, hyperactivity and/or inattention. The DSM-5 also lists emotional lability (EL; defined as low frustration tolerance, irritability and mood lability) and cognitive impairment (defined as problems on tests of attention, executive function or memory) as associated features of ADHD that support the diagnosis of ADHD (American Psychiatric Association, 2013). ADHD affects around 5% of children (Polanczyk et al., 2007, 2014), continuing into adulthood in around two-thirds of cases with a prevalence of around 2.5% (Faraone et al., 2006; Simon et al., 2009).

The cognitive impairments which characterise ADHD in both children and adults are diverse, with the greatest evidence for deficits in executive function and reaction time variability (RTV) (Bolea-Alamañac et al., 2014; Kofler et al., 2013) (see Chapter 1, Section 1.1.10 for a more detailed discussion). Executive function, referring to the completion of goal-directed tasks, includes measures of sustained attention (commonly measured as omission errors[5]) and response inhibition (commonly measured as commission errors[6]) (Frazier et al., 2004; Willcutt et al., 2005). Deficits in omission and commission errors are commonly found in both children (Willcutt et al., 2005) and adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD. Reaction Time Variability (RTV), thought to be a measure of attentional lapses, is consistently found to be higher (indicating longer and more variable reaction times) in both children (Andreou et al., 2007; Tye et al., 2013; Uebel et al., 2010) and adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD. Although commission errors are thought to be related to impulsivity and omission errors and RTV, inattention this relationship is not clear. For example previous studies have failed to find a relationship between commission errors and hyperactivity/impulsivity and omission errors and inattention (Bédard et al., 2014; Coghill et al., 2007; Kuntsi et al., 2010). It is therefore important that the relationship between these cognitive performance measures and ADHD symptoms be examined.

Although RTV has been found to be a relatively robust deficit in adults with ADHD (Kofler et al., 2013), less well investigated in adults, is the observation that individuals with ADHD show a

[5] Where a participant fails to respond when a response is required on a computerised cognitive task.
[6] Where a participant responds when a response is not required on a computerised cognitive task.

significantly greater reduction in RTV under a rewarded task condition compared to individuals without ADHD (Kuntsi et al., 2012). This is in line with the 'state regulation' theory of ADHD, which proposes cognitive deficits in ADHD to be the result of a reduced energetic state, which in turn can be manipulated in the presence of rewards or a faster event rate (Kuntsi et al., 2012; Sergeant, 2000) (see also Chapter 1, Section 1.1.10.1). Reward sensitive improvements in MRT and RTV is a relatively robust finding in children (Kofler et al., 2013). However there has been limited research into reward sensitivity in adults, despite the importance of this finding in terms of implications for lifestyle management, in order to improve function (for example it suggests individuals with ADHD to work best in fast paced, incentive driven environments).

Along with cognitive impairments the DSM-5 also lists EL as an associated feature of ADHD (American Psychiatric Association, 2013) (for a general discussion of EL see Chapter 1, Section 1.1.3). One characteristic of EL is emotional overreactivity in response to stressful events. In children, completion of a 'frustration task' (which commonly asks the child to construct a puzzle which is missing several critical pieces (Martel, 2009)) has found a greater level of ineffective emotion regulation and reduced frustration tolerance in ADHD children compared with controls (Martel, 2009; Melnick & Hinshaw, 2000; Scime & Norvilitis, 2006; Walcott & Landau, 2004). For example, children with ADHD were found to exhibit more intense emotional expression (such as slamming their fist or sighing) (Melnick & Hinshaw, 2000), or were more likely to quit the frustration task early (Scime & Norvilitis, 2006). Evidence for emotional overreactivity in adults with ADHD has been found in a recent 'real-world' study (Skirrow et al., 2014). This study used experience sampling, in which adults with ADHD and controls were asked to rate their mood in real time, multiple times a day, during a typical working week. Emotional over-reactivity (for example in anger, frustration and irritability) to perceived 'bad events' along with increased instability of frustration and irritability were found in ADHD cases compared to controls. However no study has yet investigated whether emotional overreactivity occurs during a frustration task in adults with ADHD. Given that this has been commonly found in children, such a study would indicate the developmental stability of this trait.

The first line treatment for ADHD is stimulant medication which has been found to have the highest efficacy in treating adults with ADHD (Bolea-Alamañac et al., 2014). However some patients

have concerns regarding undesirable side-effects, partial or no response, and questions regarding the long-term efficacy and adverse effects (Faraone et al., 2008; Leonard et al., 2004; Sangal et al., 2006). Patients may therefore look to alternative treatments. Omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation is one of the most studied alternative treatments for ADHD (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013).

Results from a meta-analysis and individual studies have found an increased omega 6 to omega 3 (n-6:n-3) PUFA ratio (Chen et al., 2004; Stevens et al., 1995; Germano et al., 2007; Antalis et al., 2006) and reduced blood levels of n-3 PUFA, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Hawkey & Nigg, 2014) in children and adults with ADHD compared to controls. Deficiencies in *n*-3 PUFA may lead to inflammation (Gow & Hibbeln, 2014) and alterations in serotonin and dopamine (Assisi et al., 2006; Chalon, 2006); mechanisms implicated in the pathophysiology of ADHD (Bolea-Alamañac et al., 2014; Gizer et al., 2009; Strickland, 2014).

Meta-analyses have found a small to moderate effect of *n*-3 PUFA supplementation in reducing ADHD symptoms in children with ADHD (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). We recently showed there to be little evidence for any beneficial effect of *n*-3 PUFA supplementation on cognition in children with ADHD and related disorders and typically developing children and adults (Cooper et al., 2015) (Chapter 2). We also found suggestive evidence for a small effect of *n*-3 PUFA on reducing EL and oppositional behaviour in children with ADHD (R E Cooper et al., 2016) (Chapter 3). As yet no study has investigated the effect of *n*-3 PUFA supplementation in adults with ADHD.

5.2.1 Objectives

The main aim of this pilot study was first to, investigate cognitive performance deficits (in particular reward sensitivity) and symptoms of EL (in particular emotional overreactivity during a 'frustration task') in adults with ADHD, and second to, provide preliminary data as to whether supplementation with *n*-3 PUFA improves cognition, ADHD symptoms and EL in adults with ADHD. The following hypotheses were tested: 1) That compared to controls, adults with ADHD would show impaired cognitive performance (including reward sensitivity) and increased symptoms of ADHD and EL (including emotional overreactivty) at baseline; 2) That adults with ADHD compared

to controls would have reduced plasma and red blood cell levels of *n*-3 PUFA and a higher *n*-6:*n*-3 PUFA ratio; 3) That supplementation with *n*-3 PUFA in adults with ADHD would improve cognitive performance, ADHD symptoms and, EL.

5.3 Methods

5.3.1 Participants

ADHD cases: Participants in the clinical trial were 81 adults with ADHD (44 male, 37 female, mean age = 33.5 years (10.26), research diagnosis: N=10, clinical diagnosis: N=71), 23 (28.4%) had previously been diagnosed with ADHD in childhood while the rest (N=58) were first time diagnoses in adulthood. The majority of participants had combined type ADHD (N=73) with only a small number with inattentive type (N=8).

Controls: Thirty healthy control participants (16 male, 14 female,), matched roughly in age (mean age = 29.51 years (SD = 8.80), t = -1.90 (p = 0.06)) and IQ (t = 0.83 (p = 0.41)) (although there was a trend for controls to be slightly younger than the ADHD cases) completed baseline assessments (see Table 5-1)

5.3.2 Design

In order to test hypothesis three (that supplementation with *n*-3 PUFA in adults with ADHD will improve cognitive performance, ADHD symptoms and EL) a single-centre 6-month double-blind, placebo controlled, parallel-group pilot study with balanced randomisation (1:1) was conducted. In order for hypotheses one and two to be tested, a sample of 30 typically developing control participants undertook the baseline assessments of the trial (see Figure 5-1). For hypothesis one case/control comparisons were made for measures of EL, ADHD symptoms and cognitive performance. Measures that showed at least nominally significant ($p < .05$) case/control differences were taken forward as outcome measures in the RCT analysis. For hypothesis two, case/control differences in plasma and red blood cell levels of the *n*-3 PUFAs: (EPA), (DHA), docosapentaenoic acid (DPA), alpha-linoleic acid (ALA) and the *n*-6:*n*-3 PUFA ratio were examined. These PUFAs were chosen due to previous research including a meta-analysis, finding case/control differences on these measures (reduced EPA, DHA, DPA and ALA and an increased *n*-6:*n*-3 ratio) (Antalis et al., 2006; Chen et al., 2004; Germano et al., 2007; Hawkey & Nigg, 2014; Stevens et al., 1995) (the study design is shown in Figure 5-1). The RCT analysis was presented in accordance with guidance from

the CONSORT Statement (Consolidated Standards of Reporting Trials) (Schulz, Altman, Moher, & Group, 2010)

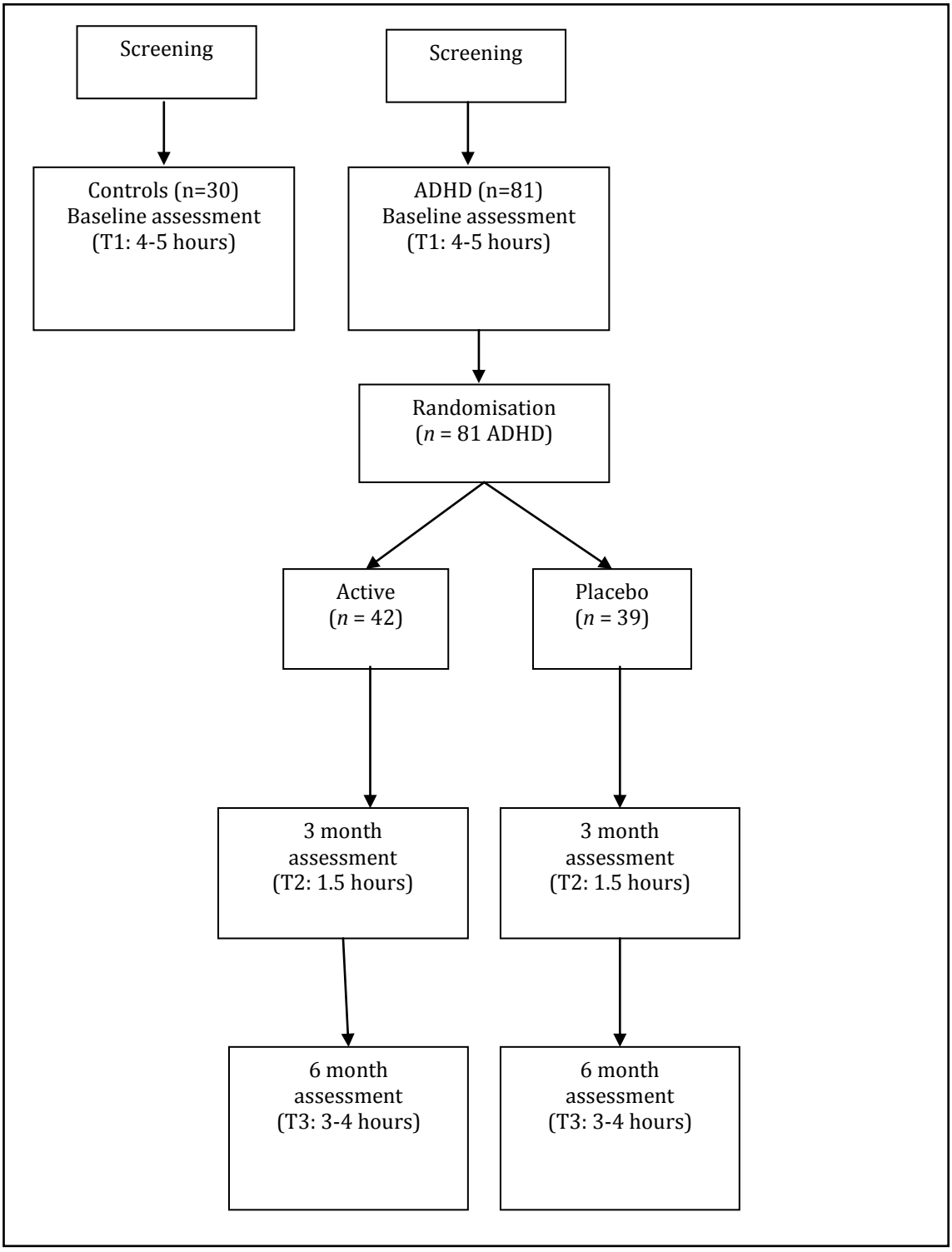


Figure 5-1: OCEAN study design

T1 = time 1, T2 = time 2, T3 = time 3

5.3.3 Inclusion and exclusion criteria

See Chapter 4, Section 4.2.2.1.

5.3.4 Study setting, funding and ethical approval

See Chapter 4, Section 4.2.1.1.

5.3.5 Supplementation

Participants in the active group were supplemented with Equazen High concentrated (Equazen HC) taken as four capsules per day of 279 mg EPA, 87 mg DHA, 30 mg GLA: stable ratio EPA:DHA:GLA (9:3:1) which equates to 1,116 mg of EPA, 348mg DHA, and 120mg GLA per day. Participants in the placebo group received four capsules each containing 648mg medium chain triglycerides (an inert fatty acid). Following completion of the trial, individuals on the placebo were offered a 6-month supply of *n*-3 PUFA supplements. The placebo was matched to the active supplement for taste, colour, and size.

5.3.6 Outcomes

Baseline only measures

- **The MINI 6.0 (Mini International Neuropsychiatric Interview) diagnostic interview** (Lecrubier et al., 1997): The MINI was used to screen for comorbid disorders.
- **The Weschler Abbreviated Scale of Intelligence – II (WASI-II)** (Weschler, 2005): Two subtests (vocabulary and matrix reasoning) of the WASI-II were used to measure IQ.
- **Socio-economic status (SES):** SES was assessed by collecting information on participants' level of education, occupation and income.
- Information regarding the participants' medication and when they were diagnosed with ADHD was also collected.

5.3.6.1 Primary outcome

Sustained Attention to Response Task (SART) (O'Connell et al., 2009; Skirrow et al., 2015)**:** The primary outcome was commission errors on the SART task (a measure of inhibition (Frazier et al., 2004)). This was considered the primary outcome as inhibitory control deficits are known to be

associated with ADHD, and a previous case/control comparison using the SART found adults with ADHD to be significantly impaired on this measure compared with controls (Skirrow et al., 2015).

The SART is a computerised go/no go task measuring both response inhibition and sustained attention. It consists of nine digits presented in random order on a computer monitor. Participants are instructed to withhold responses to the digit 3 (no-go trial) but to respond with a button press after all other digits (go trial). Participants completed the SART over three blocks, each lasting approximately 5 min. Individual blocks consisted of 225 digits, with each digit presented 25 times. Stimuli were presented in five digit sizes (font size 100, 120, 140, 160 and 180 in Arial text), subtending approximately 1.7°, 2.1°, 2.4° and 2.7° respectively in the vertical plane. Digits were presented .31° above a central white fixation cross on a grey background for 150 ms, followed by an inter-stimulus interval of 1000ms. Measures recorded were as follows: commission errors (where the participant responds where a response is not required) and omission errors (where a participant fails to respond when a response is required) were added across the three trials. For mean reaction time (MRT), reaction time variability (RTV) (as indexed by the standard deviation of reaction time) and the coefficient of variation (CV) (RTV/MRT) the average was computed across the three trials.

5.3.6.2 Secondary outcomes

ADHD symptoms:

- ***Conners' Adult ADHD Rating Scales (CAARS)*** (Conners et al., 1999) ***and Wender-Reimherr Adult Attention Deficit Disorder Scale (WRAADS)*** (Wender, 1995) ***combined (investigator rated):*** Assessed ADHD symptom severity. Total scores for each category of inattention, hyperactivity/impulsivity, and emotional lability were used in the analysis.

Emotional lability:

- ***Rating scales:*** The Centre for Neurologic Study Lability Scale (CNS-LS) (Moore et al., 1997) and Affective Lability Scale-Short Form (ALS-SF) (Oliver & Simons, 2004) measured emotional lability. Total score from each was used in the analysis.
- ***The Computerized Paced Auditory Serial Addition Task*** *(PASAT-C* (Lejuez, 2003)*)*: The PASAT is a computerised task designed to elicit frustration. Participants are asked to sum

numbers sequentially as they appear on a computer screen. The speed of the task increases over time and, with each error, negative feedback is provided (a loud noise). In order to control for cognitive ability the PASAT utilizes a titration level where participant skill level is measured during the first round which then determines the speed for the more difficult rounds. In the second round the task begins and ends with computerised rating scales where the participant was asked to rate their levels of frustration and irritability. The numbers are presented the fastest in the third round. This round times out at a set time (7 minutes) but participants have the option to quit the task at any time. In order to increase frustration levels, the following socio-evaluative components were added: 1) the participant was made aware that the task was being filmed; 2) an evaluative audience was present during the task (the experimenter) although not during ratings of frustration/irritability; 3) A negative social comparison was made as participants were informed that scores on this task were recorded and ranked against other participants. They were told that if the total number of points earned was greater than the average of the other participants they would receive a prize in the post (Dickerson & Kemeny, 2004). All participants received a £2.50 supermarket voucher in the post around 4 weeks after the testing session.

Cognition:

- **Cued Continuous Performance Test (CPT-OX) with flankers** (McLoughlin et al., 2010; Valko et al., 2009): The task consisted of 400 black letters, including cue letter 'O', target letter 'X', and distractors 'H', 'B', 'C', 'D', 'E', 'F', 'G', 'J', and 'L'. Letters were presented centrally on the computer, subtending approximately 0.5°. Letters were flanked on either side by the letters 'X' or 'O', and cue and target letters (O and X) were flanked by the incompatible letter (X and O). Participants were instructed to ignore the flanking letters and respond as quickly as possible to cue-target sequences (O-X). 80 cues (O) were followed by the target letter (X) in 40 trials (go condition), and neutral distractors in the remaining trials (no-go condition). In 40 trials a letter X was not preceded by a cue O and had to be ignored. Letters were presented every 1.65s for 150 ms in pseudo-random order. Ten practice trials preceded the main task. Task duration was 11 minutes. Omission errors, commission errors, MRT, RTV and CV were recorded.

- **The Fast Task** (Andreou et al., 2007)

The Fast Task is a computerised standard warned four-choice RT task. The baseline (slow-unrewarded) condition consisted of the following: A warning signal (four empty circles, arranged side by side) first appeared on the screen. At the end of the fore-period (presentation interval for the warning signal), the circle designated as the target signal for that trial was filled (coloured) in. The participant was asked to make a compatible choice by pressing the response key that directly corresponded in position to the location of the target stimulus. Following a response, the stimuli disappeared from the screen and a fixed inter-trial interval of 2.5s followed. Speed and accuracy were emphasized equally. If the participant did not respond within 10 seconds, the trial terminated. A practice session was first administered followed by the baseline condition, with a fore-period of 8s and consisting of 72 trials.

To investigate the extent to which a response style characterized by slow and variable speed of responding can be maximally reduced, the task includes a comparison (reward) condition that uses a fast event rate (fore-period of 1s) and incentives. This condition started immediately after the baseline condition and consisted of 80 trials and a fixed inter-trial interval of 2.5 s. Speed and accuracy were emphasized equally. The participant was told to respond really quickly one after another, to win smiley faces and earn a real prize at the end. The participant won a smiley face for responding faster than their own MRT during the baseline (first) condition consecutively for three trials. The smiley faces appeared below the circles in the middle of the screen and were updated continuously. The fast-incentive condition is always administered after the baseline condition and, as such, does not involve a similar learning phase. Participants earned a £2.50 supermarket voucher after the task battery. Measures of MRT, RTV and CV were obtained from the baseline and reward condition. Change scores were calculated as baseline minus the fast-incentive condition.

5.3.6.3 Blood PUFA analysis

In order to analyse case/control differences and monitor compliance during the trial a trained phlebotomist (myself) collected 2 x 10ml blood from controls at time 1 (baseline), and from each of the ADHD cases entering the trial at time 1 (baseline), time two (3 months), and time three (6 months). Blood was collected in lithium heparin vacutainers and taken immediately to the laboratory at the SGDP centre. Five mls of whole blood were pippeted into 1ml matrix tubes. The

remaining blood was centrifuged at 1500g for 10 minutes. Five mls of plasma were then pippeted into 1ml matrix tubes. An equivalent volume of saline (0.85% sodium chloride) was added to the remaining red blood cells and the tubes were inverted. The tubes were then centrifuged (1500g) for 10 minutes and the supernatant was discarded. This process (saline and centrifugation) was then repeated and 4mls of red blood cells were pippeted into 1 ml matrix tubes. The whole blood, plasma and red blood cells were then stored at -80°c. The blood fatty acid analysis was conducted at the National Institute of Health, Maryland, United States. Blood samples were shipped by courier in two batches. The plasma and red blood cells were assayed for total fatty-acid composition utilising a high throughput robotic direct methylation coupled with fast gas-liquid chromatography (see Lin et al., 2012 for a detailed description of this methodology). Omega-3 PUFA levels were reported in both the plasma and red-blood cells: plasma levels reflect more recent fluctuations (days) in *n*-3 PUFA while red-blood cells reflect more long-term changes (months) (Hawkey & Nigg, 2014).

5.3.7 Procedure

All participants were sent a letter by post, confirming their agreed appointment time and date. Letters included the questionnaire measures (outlined in Section 4.2.4) to be completed and brought to appointments. For the time 1 and time 3 assessments participants (ADHD cases) on stimulant medication were asked to stop taking their medication for 48 hours before the assessment, and they were also asked to refrain from drinking caffeine or smoking on the day of each study session, and to refrain from consuming alcohol on the day of the study session or during the preceding evening. Instructions to this effect were included in the appointment confirmation letter, and were also given by telephone during appointment reminders, which were either one or two days before each research appointment.

At the time 1 visit participants (ADHD cases and controls) first underwent the cognitive-electrophysiological session (which involved completion of the cognitive tasks (data not analysed here)). They then completed the IQ test and the WIAT-II (data not analysed here), before taking a 20 minute lunch break. After lunch participants completed the PASAT frustration task, the MINI and the self-report questionnaires (Section 4.2.4). Finally a blood sample was taken (Section 5.3.6.3). At the end of the baseline session the ADHD cases received a 6 month supply of either the

placebo or active supplements and received full instructions on how to take them. At the time 2 visit participants (ADHD cases) completed the same questionnaires as at baseline and gave a blood sample. At the time 3 visit participants (ADHD cases) completed an identical testing session to the time 1 visit although they did not complete the IQ test or the MINI. All participants were compensated for travel and received £20 after the time 1 and time 3 testing sessions.

5.3.8 Sample size

For the case/control comparison with a sample size of 80 cases and 30 controls and with 80% power we were able to detect a medium effect at a nominal level of significance (d=0.6, α = 0.05). The study was therefore reasonably powered to detect case/control differences for the primary outcome, commission errors on the SART which has been estimated to be at medium effect (d=0.7). We are also relatively well powered to detect differences in *n*-3 PUFA blood levels which have been generally estimated at medium effect (*d*=0.4-0.6) (Hawkey & Nigg, 2014).

As our randomised controlled trial was a pilot study, examining feasibility and trends in treatment effects, a formal power calculation was not required. However, with a total sample size of 80 participants and 80% power, we are able to detect a medium effect at a nominal level of significance (*d*=0.6, α = 0.05).

5.3.9 Randomisation

Randomisation and blinding were carried out by Vifor Pharma. The randomisation list was generated using blockwise randomisation with a fixed block size of 10 using the Randlist software (RandList®, DatInf GmbH, Tu¨bingen, Germany). Random number generation was based on the algorithm of Park and Miller with a Bays-Durham correction (Park & Miller, 1988). Two different randomisation lists were produced for males and females. Each randomisation list was double-checked to ensure there were no issues (i.e. size of block, random repetition between different arms).

5.3.9.1 Randomisation: allocation concealment mechanism

The Equazen HC and placebo were in capsule form and were identical in appearance, smell, and taste. They were pre-packed in bottles and consecutively numbered in two separate blocks for each

male and female according to the randomisation schedule. The capsules were then given out in consecutive order as each participant began the trial. The allocation sequence was kept in sequentially numbered, opaque, sealed envelopes in a locked drawer in the Social, Genetic and Developmental Psychiatry Centre.

5.3.10 Blinding

Investigators, participants and those assessing outcomes were all blind to treatment allocation. Post-intervention, participants were asked whether they believed they had been taking the active or placebo supplements and these estimates were used to assess the maintenance of blinding (although this process was initiated halfway through the study and therefore only a small number of participants (n=31) were asked to guess their group allocation). At the end of each final assessment participants were unblinded and were provided with a 6-month supply of active supplements if they had been taking the placebo.

5.3.11 Statistical methods

All analyses were completed in SAS® 9.3 Software (Statistical Analysis Software) and STATA® (StataCorp, 2009). For all analyses a nominal level of significance was set at $p < .05$. The significance level after correction for multiple testing is detailed in each of the below sections.

5.3.11.1 Case/control baseline comparisons

Comparisons of blood PUFA levels, ADHD symptoms, cognition, and emotional lability were conducted using T-tests or Mann-Whitney two sample rank sum tests (if data were non-normal). Repeated measure comparisons (for the baseline to incentive condition on the Fast Task and pre/post task scores of frustration and irritability on the PASAT) were conducted using either paired samples t-tests (normal data) or the Wilcoxon matched-pairs signed-ranks test (non-normal data). Case/control differences in change scores (baseline minus fast incentive condition) were then conducted using Mann-Whitney two sample rank sum tests (for non-normal data) to assess whether the change was greater in cases or controls (Andreou et al., 2007). Pearson or Spearman (for non-normal data) correlations were used to examine the relationship between the cognitive task data (omission/commission errors) or frustration task data and ADHD symptoms including EL. Normality for baseline comparisons was assessed by conducting the skewness and kurtosis tests

for normality (if p< .05 for both skew and kurtosis, data was considered to be non-normal) , examination of the value of skew (1 to -1 was considered normal), and inspection of the histogram. For blood PUFA levels the significance level was corrected for 12 statistical tests (Bonferroni correction set at p < .004). For ADHD symptoms, cognition, and emotional lability the significance level was corrected for 31 statistical tests (Bonferroni correction set at p ≤ .002).

5.3.11.2 RCT analysis

Examination of blood PUFA change: Changes in blood PUFA levels over the three time points during the trial were examined between the placebo and active arms by group (using SAS) and at an individual level (using Microsoft Excel). In order to assess group changes the covariance structure of the data was examined, and no discernible pattern was found. Therefore treatment effects were estimated using a linear marginal model to account for intra-patient correlation in repeated measures, with an unstructured covariance matrix (Kincaid, 2005) (the procedure MIXED was used). Linear models are robust to deviations from normality (Hamer & Simpson, 2000), and modelling the covariance structure of repeated measures is considered an effective method to handle missing data without the need for multiple imputation (MI) (Gadbury, Coffey, & Allison, 2003). A significant group x time interaction indicated a greater PUFA increase in the active over the placebo group. Where effects were significant post-hoc comparisons using the least-square means (LS-MEANS) were conducted (using the LSMEANS/PDIFF statement). LS-Means are the regression adjusted means estimated from the linear model. Least square means represent a statistical average which have been adjusted for missing data and are therefore more appropriate to use for contrasts than the raw means. The significance level was corrected for eight statistical tests (Bonferroni correction set at p ≤ 0.006). At an individual level, time 1-time 2, and time 1-time 3 changes in EPA (the main *n*-3 PUFA included in the supplements) were assessed. An increase of ≥ 50% was considered to indicate compliance (Hibbeln, personal communication).

Examination of treatment effects: The Intent-to-treat (ITT) and per-protocol analyses were conducted in SAS®. The ITT analysis included every participant who was randomised to the trial (and for whom follow-up data was available), regardless of protocol deviations, including compliance (Lewis & Machin, 1993), and was considered the primary analysis. The per-protocol analysis included only those patients who adhered to the protocol (Lewis & Machin, 1993). We

defined this as those who returned for their time 2 (for variables measured at time 1 to time 2) or time 3 (for variables measured at time 1 and time 3) assessment and who were considered compliant according to their blood PUFA (described above). This was considered the secondary analysis. For both the ITT and per-protocol analyses no discernible pattern to the covariance structure of the data was found therefore treatment effects were estimated using a linear marginal model to account for intra-patient correlation in repeated measures, with an unstructured covariance matrix (Kincaid, 2005). A significant group x time interaction indicated the presence of a treatment effect. Where effects were significant (for the variables measured at 3 time points), post-hoc comparisons using LS-MEANS were conducted (as above). Linear models are robust to deviations from normality and considered to be an effective method to handle missing data without the need for multiple imputation (MI) (Gadbury et al., 2003). Multiple imputation was therefore implemented in the ITT analysis as a sensitivity analysis only (Allison, 2012; Twisk, De Boer, De Vente, & Heymans, 2013). Multiple imputation was conducted using the PROC MI procedure in SAS using an arbitrary simulation (as data was missing in an arbitrary pattern given that drop-out occurred at two time-points) with the Fully Conditional Specification (FCS) method (which imputes data at baseline and follow-up). The imputation model included all variables in the outcome analyses. Missing data was assumed to be missing at random (MAR). The significance level was corrected for 25 statistical tests (Bonferroni correction set at $p \leq 0.002$).

5.3.12 Losses and exclusions

Blood PUFA analysis: Blood could not be obtained at baseline from four cases and one control, and at time 2, from one case. Technical problems at the PUFA processing stage meant that data was missing for one control and two cases at time 1 (one case was missing red blood cells (RBC) only) and one case at time 2 (RBC only).

Outcome measures: At baseline: one ADHD case could not tolerate the EEG and failed to complete the Fast Task, CPT and SART, eleven cases were excluded from the CPT as extreme omission or commission errors (> 3.5 SDs from the mean) indicated they had not understood the task (Skirrow et al., 2015), and one case was excluded from the SART due to extreme omission errors (> 3.5 SDs from the mean). One case failed to complete the CNS-LS and ALS at baseline. At time 3 one case was excluded from the CPT due to extreme commission errors.

5.4 Chapter outline

The remainder of this Chapter has been sectioned into two;

Part one (results, discussion) - case/control comparisons in order to meet objectives:

1) That compared to controls, adults with ADHD would show impaired cognitive performance and increased symptoms of ADHD and emotional lability.

2) That adults with ADHD compared to controls would have reduced plasma and red blood cell levels of *n*-3 PUFA and a higher *n*-6:*n*-3 PUFA ratio.

Part two (results, discussion) - RCT of *n*-3 PUFA supplementation in adults with ADHD, in order to meet objective:

3) That supplementation with *n*-3 PUFA in adults with ADHD will improve cognitive performance, ADHD symptoms and emotional lability.

5.5 Part One: A case-control comparison of cognitive performance, ADHD symptoms, emotional lability and *n*-3 PUFA blood levels in adults with ADHD compared to controls

5.5.1 Results (Part one): Hypothesis 1- Compared to controls, adults with ADHD will have impaired cognitive performance and increased symptoms of emotional lability and ADHD

Table 5-1 details the case/control comparisons. Variables that showed at least a nominally significant (at $p < .05$) case/control difference were taken forward as outcomes for the RCT analysis (as stated in Section 5.3.2). There was no significant difference in age (t=-1.90, p=.06) and IQ (t=0.83, p=.41) between cases and controls, although there was a trend for participants in the ADHD group to be older.

5.5.1.1.1 Cognitive outcomes

SART and the CPT: ADHD cases compared to controls had increased omission (OE) and commission (CE) errors on the SART (OE: t =-4.40, p<.0001; CE: t=-4.93, p<.0001) and the CPT (OE: z = -2.91, p = 0.004; CE: Z = -3.83 p = 0.0001). Cases had increased RTV and CV on the SART (RTV: z = -4.32, p <.0001; CV: z = -4.11, p <.0001) and the CPT (RTV: z = -2.81, p = 0.01; CV: z = -3.37, p = 0.0007) compared to controls. All differences withstood correction for multiple testing (p≤.002). No differences were found in MRT on the CPT and SART (p> .05) (although there was a trend for ADHD cases to have longer reaction times on the CPT (z = -1.74, p = 0.08).

Pearson correlations in ADHD cases and controls (conducted separately) found there to be no relationship between commission errors (in the SART and CPT tasks) and symptoms of hyperactivity/impulsivity and omission errors (In the SART and CPT tasks) and symptoms of inattention (all p > .05) (see Appendix D, Tables AD-1 – AD-2).

Fast task: Cases compared to controls had increased MRTs, RTVs and CVs on the Fast Task in both the baseline (MRT: z = -3.71, p = 0.0002; RTV: z=-2.98, CV: p = 0.003; z = -2.21, p=0.03) and fast-incentive (MRT: z = -2.30, p = 0.02; RTV: z=-2.91, p=0.004; CV: z=-2.58, p = 0.01) conditions. Only the baseline MRT difference withstood correction for multiple testing (p≤.002).

The fast incentive condition led to significantly reduced MRT in both ADHD cases (z=7.60, p < .0001) and controls (z=4.78, p < .0001), examination of change score (baseline – fast-incentive) showed that the reduction was greater in ADHD cases (z=-2.90, p=0.004). Significantly reduced RTV was found in both cases (z=6.77, p < .0001) and controls (z=4.78, p < .0001) with a trend for a greater reduction in cases (z=-1.72, p=0.09). Finally, CV reduced equally (p=0.83) in both cases (z=5.27, p < .0001) and controls (z=4.33, p < .0001).

5.5.1.1.2 ADHD symptoms and emotional lability

ADHD Symptoms: Cases had significantly higher symptoms of ADHD (inattention (t=-17.36, p<.0001), hyperactivity/impulsivity (t=-12.79 p<.0001) and emotional lability (t=-10.80, p<.0001) than controls, all of which withstood correction for multiple testing (p≤.002).

Emotional lability: Cases had significantly higher levels of emotional lability on the CNS-LS (t=-8.75, p < .0001) and the ALS (t=-8.84, p< .0001), which withstood correction for multiple testing (p≤.002).

PASAT: ADHD cases compared to controls had significantly higher ratings of pre (z=-3.10, p = 0.002) and post (t=-3.31, p=0.001) task frustration, and pre (z=-3.99, p = 0.0001) and post (t=-3.25, p=0.002) task irritability, all of which withstood correction for multiple testing (p≤.002). Cases chose to quit the PASAT quicker than controls (z=2.19, p = .03), indicating reduced frustration tolerance (at nominal significance).

The PASAT led to significant increases in frustration (f) and irritability (i) in both cases (f: t=13.59, p< .0001; i: t=-8.80, p< .0001) and controls (f: t=-7.35, p< .0001; i: t=-5.06, p< .0001)[7], which withstood correction for multiple testing (p≤.002). There was a trend for ratings of frustration to increase more in cases compared to controls (t=-1.80, p =.08), this trend was much weaker for ratings of irritability (t = -1.46, p = 0.15).

Spearman correlations in ADHD cases found there to be a significant positive relationship between post-task ratings of irritability (r_s = 0.29, p =0.008), frustration (r_s =0.45, p < .0001), and EL rated with the CAARS/WRAADS. There was also a significant positive relationship between post-task frustration and the CNS-LS (r_s=0.30, p =0.006). In cases there was no relationship between post-task irritability and the CNS-LS and ALS or between post-task frustration and the ALS (all p> .05). In controls there was no relationship between post-task ratings of irritability and frustration and EL measured with the CAARS/WRAADS, the CNS-LS and the ALS (all p>.05) (see Appendix D, Tables AD-3 – AD-4).

[7] for this analysis as the pre-test variables were non-normal and the post-test normal, both parametric and non-parametric tests were run with identical 'p' values therefore I report the parametric analysis

Table 5-1: Case/control comparisons of outcome measures

	N ADHD	**N Control**	**ADHD *M*(SD)**	**Control *M*(SD)**	**t(*p*)**	**MW *z(p)***	**Cohen's *d***
Age	81	30	33.52 (10.26)	29.51 (8.80)	-1.90 (0.06)	-	-
IQ	80	30	109.38 (13.68)	111.70 (11.44)	0.83 (0.41)	-	-
Cognition							
SART CE	79	30	34.48 (12.80)	21.27 (11.69)	-4.93 (<.0001)**	-	1.07
SART OE	79	30	23.09 (27.01)	10.17 (23.05)	-	-4.40 (<.0001)**	0.5
SART RTV	79	30	132.86 (62.28)	85.41 (33.22)	-	-4.32 (<.0001)**	0.86
SART MRT	79	30	327.40 (52.45)	310.30 (40.33)	-	-1.36 (0.17)	0.35
SART CV	79	30	0.41 (0.18)	0.28 (0.13)	-	-4.11 (<.0001)**	0.78
CPT CE	68	30	2.94 (2.78)	1.07 (1.36)	-	-3.83 (0.0001)**	0.77
CPT OE	68	30	1.87 (2.23)	0.83 (2.44)	-	-2.91 (0.004)*	0.46
CPT MRT	68	30	408.93 (65.81)	387.02 (51.47)	-	-1.74 (0.08)	0.36
CPT RTV	68	30	139.60 (64.65)	109.40 (78.53)	-	-2.81 (0.01)*	0.44
CPT CV	68	30	0.34 (0.14)	0.25 (0.17)	-	-3.37 (0.0007)**	0.61
Fast task MRT	80	30	713.15 (246.62)	561.27 (112.96)	-	-3.71 (0.0002)**	0.7
Fast task RTV	80	30	206.04 (192.53)	132.11 (88.14)	-	-2.98 (0.003)**	0.44
Fast task CV	80	30	0.26 (0.13)	0.22 (0.11)	-	-2.21 (0.03)*	0.32
Fast task reward MRT	80	30	514.63 (146.81)	450.76 (69.66)	-	-2.30 (0.02)*	0.49
Fast task reward RTV	80	30	111.24 (72.59)	79.29 (45.36)	-	-2.91 (0.004)*	0.49
Fast task reward CV	80	30	0.20 (0.07)	0.17 (0.07)	-	-2.58 (0.01)*	0.43

	N ADHD	N Control	ADHD *M*(SD)	Control *M*(SD)	t(*p*)	MW *z(p)*	Cohen's *d*
Fast task change MRT[a]	80	30	198.52 (176.61)	110.51 (67.80)	-	-2.90 (0.004)*	0.57
Fast task change RTV[a]	80	30	94.80 (172.86)	52.82 (59.17)	-	-1.72 (0.09)	0.28
Fast task change CV[a]	80	30	0.06 (0.12)	0.06 (0.07)	-	-0.22 (0.83)	0.00
ADHD Symptoms							
CW Inattention	81	30	27.16(6.13)	6.23 (3.99)	-17.36 (<.0001)**	-	3.74
CW Hyp/Imp	81	30	20.09 (5.80)	5.33 (4.08)	-12.79 (<.0001)**	-	2.76
CW EL	81	30	17.99 (7.13)	3.33 (3.36)	-10.80 <.0001)**		2.33
Emotional lability							
CNS-LS	80	30	32.75 (16.49)	5.67 (6.12)	-8.75 (<.0001)**	-	1.89
ALS	80	30	24.31 (12.09)	4.17 (4.86)	-8.84 (< .0001)**	-	1.91
PASAT time to quit	81	30	325.64 (143.47)	378.43 (110.36)	-	2.19 (0.03)*	0.39
PASAT Frustration pre-task	81	30	12.64 (18.77)	2.77 (2.80)	-	-3.10 (0.002)**	0.62
PASAT Frustration post-task	81	30	62.81 (32.53)	40.67 (27.81)	-3.31 (0.001)**	-	0.71
PASAT Irritability pre-task	81	30	13.80 (20.70)	2.67 (3.77)	-	-3.99 (0.0001)**	0.63
PASAT Irritability post-task	81	30	48.14 (32.61)	26.70 (25.60)	-3.25 (0.002)**	-	0.7
PASAT Frustration change[b]	81	30	50.17 (33.24)	37.90 (28.23)	-1.80 (0.08)	-	0.39
PASAT Irritability change[b]	81	30	34.33 (35.10)	24.03 (26.03)	-1.46 (0.15)	-	0.32

Note. CE = Commission errors, OE = Omission errors, a. Change scores calculated as baseline minus fast-incentive

b. Change scores calculated as post (irr/frust) minus pre (irr/frust), *Significant at nominal level ($p \leq .05$), **Significant after correction for multiple testing ($p \leq .002$)

5.5.2 Hypothesis 2: Adults with ADHD compared to controls will have reduced *n*-3 PUFA and a higher *n*-6:*n*-3 PUFA ratio

Case/control comparisons found no difference in red blood cell (RBC) or plasma levels of total *n*-3 PUFA, EPA, DHA, ALA or *n*-6:*n*-3 PUFA ratio ($p > .05$). ADHD cases had significantly higher levels of the *n*-3 PUFA DPA in RBC's ($t=-3.15$, $p = 0.002$) and plasma ($t=-2.02$, $p = 0.05$) (the latter was nominally significant) (see Table 5-2)

Table 5-2: Blood PUFA comparison between cases and controls

Measure	**N ADHD**	**N Controls**	**ADHD *M*(SD)**	**Control *M*(SD)**	**t(*p*)**	**MW *z(p)***	**Cohen's *d***
Plasma							
Total n-3 HUFA µg/mL	76	28	63.32 (34.27)	52.91 (32.46)	-1.39 (0.17)	-	0.31
EPA (20:5n3 µg/ml)	76	28	19.51 (11.68)	16.00 (12.61)	-	-1.69 (0.09)	0.3
DHA (22:6n3 µg/ml)	76	28	31.52 (19.39)	27.51 (16.43)	-0.97 (0.33)	-	0.22
ALA (18:3n3 µg/ml)	76	28	25.77 (11.84)	24.60 (10.65)	-0.46 (0.65)	-	0.1
DPA (22:5n-3 µg/ml)	76	28	12.29 (6.55)	9.39 (6.43)	-2.02 (0.05)*	-	0.45
Σ *n*-6: Σ *n*-3 PUFA	76	28	3.84 (1.41)	4.45 (2.04)	-	1.12 (0.26)	0.38
Red blood cells							
Total *n*-3 HUFA µg/g	75	28	88.36 (25.08)	79.22 (22.84)	-1.69 (0.10)	-	0.38
EPA (20:5n3 µg/g)	75	28	11.19 (4.56)	9.86 (5.52)	-1.24 (0.22)	-	0.28
DHA (22:6n3 µg/g)	75	28	47.17 (16.19)	44.47 (12.52)	-	-0.38 (0.71)	0.18
ALA (18:3n3 µg/g)	75	28	2.28 (1.02)	2.09 (0.86)	-0.89 (0.38)	-	0.2
DPA (22:5n-3 µg/g)	75	28	30.00 (7.28)	24.88 (7.48)	-3.15 (0.002)*	-	0.7
Σ *n*-6: Σ *n*-3 PUFA	75	28	2.65 (0.55)	2.98 (1.09)	-	0.76 (0.45)	0.45

* Significant at nominal level ($p \leq .05$), Σ = Total

5.6 Discussion (PART A)

5.6.1 Compared to controls, adults with ADHD will show impaired cognitive performance and increased symptoms of ADHD and emotional lability.

As expected, adults with ADHD compared to controls had impaired cognitive task performance (increased omission and commission errors, RTV, MRT and CV) and significantly higher levels of ADHD symptoms and emotional lability. Given that alongside the core symptoms of inattention and hyperactivity/impulsivity, the DSM-5 also lists emotional lability and cognitive impairment as associated features of ADHD (that support the diagnosis of ADHD) (American Psychiatric Association, 2013), this illustrates that the sample used in this study appear to be representative of typical ADHD clinic patients and therefore generalisable to typical ADHD clinical populations.

Adults with ADHD in this sample showed characteristic deficits in cognitive performance compared with controls. This was indexed by increased commission errors (the inability to withhold a prepotent response) and omission errors (failing to respond when a response is required) on the SART and the CPT task, longer mean reaction times (MRT) on the Fast Task, and greater RTVs and greater CVs (RTV controlling for MRT) on the SART, CPT, and the Fast Task.

A number of studies have reported increased commission and omission errors on the same or similar tasks in both children (Willcutt et al., 2005) and adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD (for reviews see Bolea-Alamañac et al., 2014; Frazier et al., 2004). It has been proposed that commission errors reflect impulsivity, whereas omission errors reflect impaired ability in focused and sustained attention (Frazier et al., 2004). However correlations in both cases and controls between omission errors and symptoms of inattention and commission errors and hyperactivity/impulsivity are non-significant. Therefore the data here does not support this proposal. Indeed, previous studies do not find any specific relationship between commission errors and hyperactivity-impulsivity, and omission errors and inattention (Bédard et al., 2014; Coghill et al., 2007; Kuntsi et al., 2010). Furthermore, it has been widely assumed that cognitive deficits seen in ADHD such as commission errors, omission errors, and RTV have a direct causal role to play in the generation of the ADHD symptoms of inattention and hyperactivity/impulsivity (Cheung et al., 2015). However, many different mechanisms may be responsible for change in cognitive

performance and change in behavioural symptoms (Coghill et al., 2007). For example, studies investigating the clinical response to methylphenidate have found a dissociation of the treatment effects on ADHD symptoms and cognitive performance in children and adolescents with ADHD (Bédard et al., 2014; Coghill et al., 2007; K. P. Schulz et al., 2014).

The impairments in RTV, MRT, and CV reported here are commonly found in children (Andreou et al., 2007; Tye et al., 2013; Uebel et al., 2010) and adults (McLoughlin et al., 2010; Skirrow et al., 2015) with ADHD (for reviews see Frazier et al., 2004; Kofler et al., 2013). This indicates this sample to have slower and more variable performance compared to the control group. Increased RTV is one of the most investigated and consistent cognitive performance deficits in ADHD research (Kofler et al., 2013; Kuntsi & Klein, 2011). Although slower MRTs are also found in ADHD (Lipszyc & Schachar, 2010) these differences are thought to be driven by the influence of RTV (Kofler et al., 2013). Increased RTV is thought to represent intraindividual variability in reaction time responses, described as an increase in moment-to-moment (within-subject) fluctuations in task performance (Tamm et al., 2012). Increased intraindividual variability in cognitive task performance may be due to occasional lapses in attention as a result of 'poor state regulation' (Leth-Steensen, Elbaz, & Douglas, 2000; Russell et al., 2006; van der Meere, 2002). The 'state regulation' theory of ADHD proposes the cognitive deficits in ADHD to be the result of a reduced energetic state. This theory further proposes that cognitive performance can be manipulated in the presence of rewards or a faster event rate (Kuntsi et al., 2012; Sergeant, 2000).

In line with the 'state regulation' theory, introduction of a fast-incentive condition in the Fast Task led to significant reductions of RTV, MRT, and CV in both cases and controls, with cases showing a significantly greater reduction of MRT and a trend for a greater reduction of RTV. In agreement with these findings, several studies in ADHD children have reported a significantly greater improvement in MRT and RTV following the introduction of the rewards and or an increased presentation rate (Andreou et al., 2007; Cheung et al., under review; Slusarek et al., 2001; Uebel et al., 2010). Meta-analysis has estimated a small but significant effect (g=0.3-0.4) of incentives in reducing RTV (Kofler et al., 2013), although the Fast Task was designed to maximise these improvements by combining rewards with an increase in presentation rate. In line with our

findings, Andreou et al also reported the greatest reductions for MRT but no difference in the reduction of CV.

To our knowledge, this is the first time the effects of reward and presentation rate of stimuli has been reported in adults with ADHD, suggesting that this is a developmentally stable deficit seen in both children and adults with ADHD. This sensitivity to the fast-incentive condition on the Fast Task shows the malleability of RTV and MRT, which is thought to reflect alterations in arousal or 'state regulation' and their impact on attentional fluctuations (Banaschewski et al., 2003). In accordance with the 'state regulation' hypothesis the fast-rewarded condition served to optimise our participant's 'energetic' state (Andreou et al., 2007; Konrad, Gauggel, Manz, & Schöll, 2000). Recently, it was reported that while sensitivity to the fast-rewarded condition was seen on the fast task in children with ADHD, this was not observed in a sample of children with autism, suggesting that this finding may be specific to ADHD (Tye et al., under review). Our results therefore emphasise the advantages of incorporating fast-paced activities and incentives into the environment of both children and adults with ADHD (Cheung et al., under review.), and suggest that this might reflect a developmentally stable deficit with some specificity to ADHD. Further studies are needed to rigorously evaluate the causal role of state regulation factors in ADHD across the lifespan.

As expected, increased levels of emotional lability including self (CNS-LS, ALS) and investigator-rated scales (CAARS/WRAADS measure of EL) were seen in the adult ADHD cases compared to controls. In addition, frustration and irritability ratings on a computerised mental arithmetic task designed to elicit frustration (PASAT-C) showed case-control effects. For the PASAT-C, pre and post task ratings of frustration and irritability were significantly higher in ADHD cases compared with controls. The task led to significant (pre to post task) increases in ratings of irritability and frustration in both cases and controls, with a trend for greater increases in frustration (and a weaker trend for irritability) in the ADHD group. ADHD cases also chose to 'quit' the frustration task quicker than controls, indicating a reduced ability to tolerate frustration. This suggests that along with increased self-ratings of frustration and irritability, adults with ADHD show emotional over-reactivity to stressful events under experimental conditions, and a reduced ability to tolerate these negative emotions.

To our knowledge only one previous study has used the PASAT-C in adults with ADHD. Krause-Utz et al., (2013) compared pre and post task ratings of self-reported stress in a small sample (n=15) of adults with ADHD compared to controls, as well as a sample of adults with Borderline Personality Disorder. Significant increases in pre to post-task stress in both ADHD cases and controls were found which is somewhat in line with the current results. However given that the current study measured 'frustration' and 'irritability' and Krause-Utz et al., 'stress', it is difficult to compare the two findings. Furthermore Krause-Utz et al examined neither the change in stress levels nor the time taken to quit the task between ADHD cases and controls. Aside from this study to our knowledge there is no other research using frustration tasks in adults with ADHD. Therefore the current findings of potential difficulties in emotion regulation and reduced frustration tolerance in adults with ADHD are novel.

The current results are in line with findings in children with ADHD. A number of studies, which have asked ADHD cases and controls to complete a 'frustrating puzzle' task, have found children with ADHD to exhibit more ineffective emotion regulation and reduced frustration tolerance compared with controls (Martel, 2009; Melnick & Hinshaw, 2000; Scime & Norvilitis, 2006; Walcott & Landau, 2004). For example, children with ADHD were found to exhibit more intense emotional expression (such as slamming their fist or sighing) (Melnick & Hinshaw, 2000), or were more likely to quit the frustration task early (Scime & Norvilitis, 2006). This is in line with the current finding of emotional overreactivity/emotion regulation deficits and reduced frustration tolerance in adults with ADHD. Therefore the current results provide preliminary evidence for the stability of these traits over time.

We also found significant positive correlations in ADHD cases, but not controls, between post-task ratings of irritability and frustration and investigator (CAARS/WRAADS) and self-report (CNS-LS) ratings of emotional lability (the latter correlated with post-task frustration only). This suggests these objective reactions to the frustration task map onto more subjective behavioural measures. This finding relates closely with a previous 'real-world' study using experience sampling, in which adults with ADHD and controls were asked to rate their mood in real time, multiple times a day, during a typical working week (Skirrow et al., 2014). Emotional over-reactivity (for example in

anger, frustration and irritability) to perceived 'bad events' along with increased instability of frustration and irritability were found in ADHD cases compared to controls. Here we have replicated these findings under experimental conditions, providing objective evidence for increased emotional lability and difficulties in emotion regulation in adults with ADHD. We therefore propose that the PASAT-C could be used as an objective test for emotional lability in adults with ADHD.

One limitation to our findings here is that we had over double the number of ADHD cases compared with controls. This imbalance may have inflated differences in the cases compared with controls. This potential inflation of effect size must be taken into account when interpreting our results.

In conclusion the ADHD participants included in this treatment trial, along with deficits of inattention and hyperactivity/impulsivity, showed the expected impairments in emotional lability (both self-rated and during an experimental task) and cognitive performance. The ADHD participants included in this trial are therefore representative of the general ADHD population as defined by the DSM-5, which along with the core symptoms of inattention and hyperactivity/impulsivity, also lists EL and cognitive impairment as associated features of ADHD that support the diagnosis of ADHD (American Psychiatric Association, 2013). The outcome measures which showed these case/control differences were examined as outcomes in the treatment trial. We have further reported two novel findings; the effect of incentives and stimulus presentation rate in the Fast Task, and emotional over-reactivity and reduced ability to tolerate frustration using an experimental paradigm. These or similar findings have been reported in children but not adults before. We therefore show these traits to be developmentally stable and persistent.

5.6.2 Adults with ADHD compared to controls will have reduced *n*-3 PUFA and a higher *n*-6:*n*-3 PUFA ratio

This study failed to find evidence that adults with ADHD had significantly different baseline levels of blood *n*-3 PUFA (total *n*-3 PUFA, EPA, DHA, ALA and *n*-6:*n*-3 ratio) than controls. The only significant finding was of higher levels of the *n*-3 PUFA DPA in both the RBCs and plasma (albeit nominally significant plasma levels). Therefore adults with ADHD appear to have similar, if not slightly higher, levels of blood *n*-3 PUFA than controls.

This result goes against a recent meta-analysis (Hawkey & Nigg, 2014) and individual studies (Chen et al., 2004; Stevens et al., 1995; Germano et al., 2007; Antalis et al., 2006) which have found reduced blood levels of *n*-3 PUFA (particularly EPA and DHA), and an increased *n*-6:*n*-3 PUFA ratio, in children and adults with ADHD compared to controls.

One explanation for our findings is that there may not be *n*-3 PUFA differences in adults with ADHD compared to controls, at least in this UK population. The meta-analysis which found significant differences in *n*-3 PUFA levels did so across children and adults with ADHD and did not provide separate estimates for the two groups (Hawkey & Nigg, 2014). Due to developmental effects and heterogeneity between samples, the analysis could also have been presented separately for children and adults (as was carried out in Chapter 2 (Cooper et al., 2015)). Only three of the studies included in this meta-analysis were conducted in adults (Antalis et al., 2006; Laasonen, Hokkanen, Leppämäki, Tani, & Erkkilä, 2009b; Young, Maharaj, & Conquer, 2004) with the remaining six in children (Chen et al., 2004; Colter, Cutler, & Meckling, 2008; Joshi et al., 2006; Spahis et al., 2008; Stevens et al., 1995; Stevens et al., 2003). Examination of the significance of individual effect sizes of the studies conducted in adults showed them to be at trend (p=.06-0.11), whereas three of the studies in children were significant (p=.006 -.05). Given these trend effects of *n*-3 PUFA differences in previous studies of adults with ADHD, and the negative results in this study, it is possible that a sub-analysis of studies conducted only in adults (including the current findings) may give an overall non-significant effect. Furthermore an increased *n*-6:*n*-3 ratio has mainly been found in studies in children (e.g. Chen et al., 2004; Stevens et al., 1995; Germano et al., 2007) rather than adults (only Antalis et al., 2006 appears to have examined this). Therefore *n*-3 PUFA differences appear to be more likely to be seen in children than adults with ADHD.

The suggestion of *n*-3 PUFA differences as more apparent in children than adults may question the proposed causal relationship between symptoms of ADHD and *n*-3 PUFA levels. It may be the case that due to differences in eating habits, children consume diets that are lower in *n*-3 PUFA than adults, and that this is a separate factor to symptoms of ADHD. However this is not supported by meta-analytic data, which has suggested a significant effect of *n*-3 PUFA supplementation in reducing symptoms of ADHD in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-

Barke et al., 2013). It may be that *n*-3 PUFA deficiencies are one factor that contributes towards ADHD symptom severity in childhood. Given that some but not all (~60%) of ADHD cases persist into adulthood (Faraone et al., 2006), *n*-3 PUFA deficiency may not be one of the factors that contributes to adult ADHD. Another possible explanation that is potentially linked is the recent controversial suggestion that in at least some cases adult ADHD may be a different disorder from childhood-onset forms of the condition (Moffitt et al., 2015). These are all potential explanations and future work should aim to test these hypotheses.

In further support of the negative case/control differences found in the current sample is the fact that this study has examined *n*-3 PUFA case/control differences in adults with ADHD in a much larger sample (n=76) than has been previously investigated in this population (samples included 12-36 cases) (Antalis et al., 2006; Laasonen, Hokkanen, Leppämäki, Tani, & Erkkilä, 2009a; Young et al., 2004). This should have increased not decreased the chance of finding a significant effect. Furthermore, as discussed in Section 5.6.1, we had over double the number of ADHD cases compared with controls. Such an imbalance would have been expected to inflate differences rather than mask them. Therefore the finding of a negative effect, despite the larger sample size which was weighted towards ADHD cases, increases the reliability of these null results.

An alternative explanation is that our sample was biased towards those with a high *n*-3 PUFA intake at baseline. Given that this study was an intervention study participants who were motivated to take part may have had a prior interest in *n*-3 PUFA as an ADHD treatment and may have therefore had higher levels of omega-3 consumption at baseline. The previous three studies that found a trend for reduced *n*-3 PUFA were cross-sectional studies, which may have been less prone to biased samples. In support of this the only significant finding was that ADHD cases had higher levels of the *n*-3 PUFA DPA. Although we tried to limit the recruitment of a sample who already had high *n*-3 PUFA levels (one of the exclusion criteria to the study was consumption of *n*-3 or *n*-6 supplements in the previous 3 months), we perhaps should have also included limitations on dietary fish consumption.

In summary, we did not find evidence for reduced *n*-3 PUFA in ADHD cases compared to controls. Instead we found an indication that adults with ADHD had higher *n*-3 PUFA than controls. This

suggests that deficiencies in *n*-3 PUFA may be more prevalent in a childhood rather than an adult sample, and that *n*-3 PUFA may not lie on the causal path in adult ADHD. An alternative explanation, in light of the slightly higher *n*-3 PUFA levels, could be that the null results here are a product of selection bias, with participants with high *n*-3 PUFA intake entering the study. In order to clarify results, future large cross-sectional case/control comparison studies, and subsequent meta-analyses need to be conducted. Longitudinal studies examining *n*-3 PUFA levels and ADHD symptoms from childhood to adulthood will also be required in order to examine any causal models of *n*-3 PUFA and ADHD symptoms.

5.7 Part two: A randomised controlled trial of *n*-3 PUFA supplementation in adults with ADHD

5.7.1 Results (part two): Hypothesis 3 - Supplementation with *n*-3 PUFA in adults with ADHD will improve ADHD symptoms, emotional lability, and cognition

5.7.1.1 Participant flow

Participant flow through the study is shown in Figure 5-2, and detailed reasons for exclusions at the screening stage are shown in Figure 4-2 and Appendix C, Table AC-4. Of the 1616 participants assessed for eligibility 1272 were excluded due to: not meeting inclusion criteria (n=877), did not respond to invitation to participate (n=356), not enough information to contact or screen (n=86), recruited then declined (n=14), declined to participate (n=163) or other reasons (n=39) (most commonly due to potential participants having been already recruited to other research projects or undergoing another treatment such as cognitive behavioural therapy (CBT) (n=14).

We randomised 81 participants (42 = active, 39 = placebo) into the trial. By the second follow-up (3 months), eleven participants had dropped-out of the active group, and two had dropped out of the placebo group. For the active group we lost contact with 4 participants, 3 cancelled multiple times before losing contact, one could not tolerate the EEG session and did not wish to return, one did not take the supplements, and 2 experienced personal difficulties. For the placebo group we lost contact with 1, and 1 cancelled multiple times before failing to return. Therefore 31 participants in the active and 37 in the placebo groups completed time 2 assessments. There was a significantly higher number of drop-outs in the active than placebo group before time 2 ($x^2 = 6.66$, $p = .01$).

Before the time 3 (6 month) follow-up, 5 participants had dropped out of the active group and 8 participants in the placebo group. For the active group we lost contact with one participant due to their number being disconnected, 2 had personal difficulties, 1 experienced migraines attributed to the supplements, 1 could not tolerate being medication free for 48 hours before the testing session and declined to return. For the placebo group, we lost contact with 5 participants: 1 left the country, the number was disconnected for 1 participant, and 1 cancelled multiple times before failing to return for their assessment. Therefore 26 participants in the active and 29 in the placebo completed the final (time 3) assessment. There was no significant difference in drop-outs between the placebo and active groups from time 2 to time 3. There was no significant difference in the overall number of drop-outs between the placebo (n=10) and active group (n=16) and no difference in ADHD symptom severity between drop-outs and non-drop-outs (see Appendix D, Table AD-5 – AD-6). Drop-outs were therefore assumed to be missing at random (MAR).

5.7.1.2 Numbers analysed

In the blood PUFA analysis numbers analysed were as follows: at baseline for plasma 76 cases and for RBC 75 cases, at time 2, 67 cases for plasma and 65 for RBC and at time 3, 55 cases for both plasma and RBC's.

For the primary outcome (commission errors on the SART) the ITT analysis included 26 participants in the active group and 29 in the placebo group. The per-protocol analysis (excluding nine participants from the active group whose blood PUFA levels were indicative of non-compliance) included 17 participants in the active group and 29 in the placebo group.

5.7.1.3 Recruitment

Participants were recruited between June 2012 and March 2014 and testing sessions ran from October 2012 to November 2014. Participants attended testing sessions at baseline, time 2 (3 months) and time 3 (6 months).

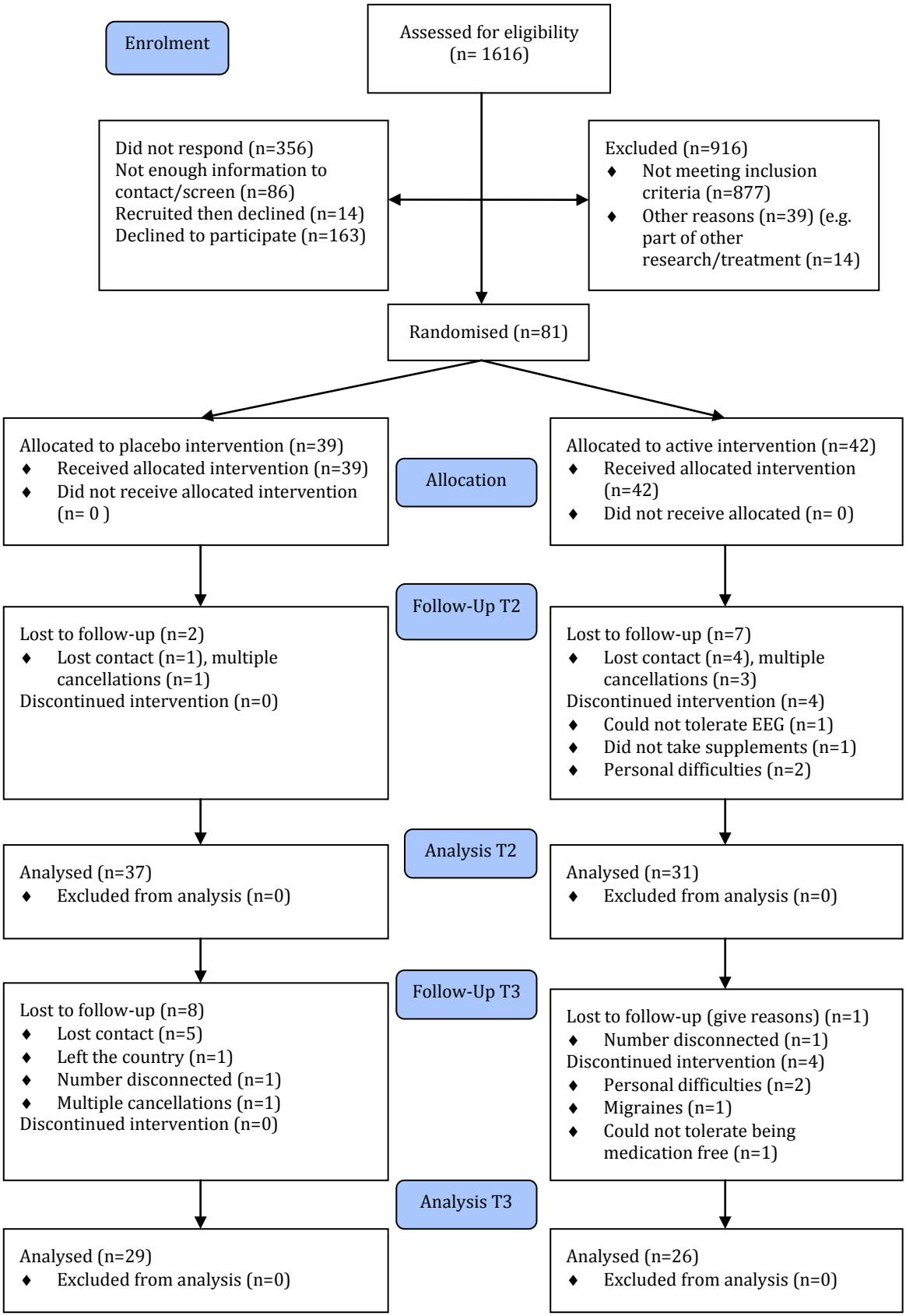


Figure 5-2: CONSORT flow diagram for the OCEAN study
Note. T2 = time 2, T3 = time 3

5.7.1.4 Baseline data

The baseline demographics for the placebo and active groups are shown in Table 5-3. There were no significant differences between the groups on age, sex, IQ, medication status (medicated/unmedicated), income, employment status, education level, or presence of comorbid mental health conditions. Table 5-4 shows baseline comparisons for all primary and secondary outcome measures. The active group had a significantly higher RTV and CV on the baseline level of the fast task than the placebo group ($p < .05$) (this is potentially a chance finding, given the large number of statistical tests that were conducted, and was only nominally significant). No differences were found in any of the other baseline measures ($p > .05$).

Table 5-3: Baseline demographics

	N A/P	**Active**	**Placebo**	**t(*p*)**	**MW *z(p)***	***X*²(*p*)**
Age (years, months) (M(SD))	42/39	33.97 (9.68)	33.04 (10.96)	-0.40 (0.69)	-	-
Sex (female/male)(n)	42/39	19/23	18/21	-	-	0.01 (0.93)
IQ (M(SD))	41/39	110.56(13.21)	108.13(14.22)	-0.79(0.43)	-	-
Medication status[a] (medicated/unmedicated) (n)	42/39	28/14	27/12	-	-	0.06(0.81)
Income[c] (M(SD))	33/31	18,718.79 (17,482.54)	19,736.80 (20,621.22)	-	- 0.01(0.99)	-
Employed (n)	42/39	27	22	-	-	0.52(0.47)
Unemployed (n)	42/39	8	11	-	-	0.94(0.33)
Full-time student (n)	42/39	7	6	-	-	0.02(0.88)
Highest level of education	42/39					
GCSE/Vocational	42/39	12	12	-	-	0.05(0.83)
AS-Level/A-Level	42/39	12	11	-	-	0.001(0.97)
Degree (undergraduate/postgraduate)	42/39	18	16	-	-	0.03(0.87)
1 or more comorbid condition (MINI)[b]	42/39	33	26	-	-	1.45(0.23)

Note. MW = Mann-whitney two sample rank-sum test, N A/P = Number of participants in active/placebo group.

a. A breakdown of medication status is available in Appendix D, Table AD-7.

b. A breakdown of comorbid disorders is available in Appendix D, Table AD-8.

Table 5-4: Baseline outcome measure comparison between the placebo and active groups in measures that showed a case/control difference.

	N A/P	Active *M*(SD)	Placebo *M*(SD)	t(*p*)	MW *z(p)*
Primary outcome					
SART RTV	40/39	129.24 (62.72)	136.58 (62.42)	-	0.47 (0.64)
Secondary outcomes					
Cognition					
SART CE	40/39	34.73 (12.84)	34.23 (12.92)	-0.17 (0.87)	-
SART OE	40/39	20.88 (20.02)	25.36 (32.80)	-	0.005 (0.996)
SART CV	40/39	0.40 (0.19)	0.41 (0.16)	-	0.46 (0.65)
CPT CE	36/35	3.11 (3.40)	4.11 (4.87)	-	0.38 (0.71)
CPT OE	36/35	1.81 (2.30)	2.09 (2.16)	0.53 (0.60)	-
CPT RTV	36/35	137.36 (59.25)	142.36 (67.96)	-	0.23 (0.82)
CPT CV	36/35	0.34 (0.13)	0.35 (0.14)	0.32 (0.75)	-
Fast task MRT	41/39	759.73 (277.59)	664.19 (201.32)	-	-1.58 (0.11)
Fast task RTV	41/39	241.89 (236.40)	168.35 (124.07)	-	**-1.96 (0.05)***
Fast task CV	41/39	0.29 (0.15)	0.24 (0.10)	-	**-2.26 (0.02)**
Fast task reward MRT	41/39	520.91 (169.96)	508.03 (119.63)	-	0.33 (0.74)
Fast task reward RTV	41/39	112.04 (78.52)	110.40 (66.81)	-	-0.05 (0.96)
Fast task reward CV	41/39	0.20 (0.07)	0.20 (0.08)	-	-0.01 (0.99)
Fast task change MRT	41/39	238.81 (201.57)	156.16 (135.95)	-	-1.78 (0.08)
ADHD Symptoms					
CW Innattention	42/39	27.10 (6.79)	27.23(5.42)	0.10 (0.92)	-
CW Hyp/Imp	42/39	20.17 (5.45)	20.00(6.22)	-0.13 (0.90)	-
CW EL	42/39	17.79 (7.20)	18.21 (7.15)	0.26(0.79)	-
Emotional lability					
CNS-LS	41/39	32.37 (16.74)	33.15 (16.42)	0.21 (0.83)	-
ALS	41/39	24.51 (11.24)	24.10 (13.07)	-0.15 (0.88)	-
PASAT time to quit	42/39	338.76 (135.11)	311.51 (152.46)	-	-0.54 (0.59)
PASAT Frustration pre-task	42/39	10.93 (17.18)	14.49 (20.40)	-	1.25 (0.21)
PASAT Frustration post-task	42/39	63.05 (32.02)	62.56 (33.49)	-0.07 (0.95)	-
PASAT Irritability pre-task	42/39	13.17 (21.61)	14.49 (19.93)	-	1.31 (0.19)
PASAT Irritability post-task	42/39	47.02 (33.11)	49.33 (32.46)	0.32 (0.75)	-

Note. N A/P = Number of participants in active/placebo group, OE = Omission errors, CE = Comission errors.

*Significant at nominal level ($p \leq .05$).

5.7.1.5 Blood PUFA changes

Changes in Blood PUFA levels over the three time points are shown in Table 5-5. At baseline there was no difference between the placebo and active group in any of the *n*-3 (EPA and DHA), or *n*-6 PUFAs (GLA), or in the *n*-6:*n*-3 PUFA ratio (all at $p > .05$), in either the plasma or red-blood cells. In plasma a significant increase in the active over placebo group was found for EPA ($p< .001$). Post-hoc analysis showed the active compared to placebo group to have significantly higher levels of plasma EPA at time 2 (t=-5.69, p=<.0001) and time 3 (z=-4.03, p=0.0001). Examination of change over time showed there to be no change in the placebo group ($p > .05$) but a significant increase in the active group from time 1 to time 2 (t = -7.61, $p < .0001$), and time 1 to time 3 (t=-5.20, p = < .0001), with a decrease from time 2 to time 3 (t=2.46, p = 0.02). A significant decrease in the total *n*-6:*n*-3 ratio was found in the active compared with placebo group ($p< .001$). Post-hoc analysis showed the active group to have a significantly lower ratio at time 2 (t=7.35, $p < .0001$) and time 3 (t=4.34, p=0.0001). Examination of the change showed there to be no change in the *n*-6:*n*-3 ratio in the placebo group but a significant reduction in the active group from time 1 to time 2 (t=7.87, $p<$ .0001) and time 1 to time 3 (t = 5.12, $p < .0001$), with a slight increase from time 2 to time 3 (t=-2.76, p =0.007).

For the red blood cells, a significant increase in the active over the placebo group was found for EPA ($p < .0001$). Post-hoc analysis showed the active compared to placebo group to have significantly higher levels of EPA at time 2 (z=-5.71, $p < .0001$), and time 3 (z=-4.38, $p < .0001$). Examination of change showed a significant reduction of EPA in the placebo group from time 1 to time 3 (t=1.95, p = 0.05) but no change between the other time points. For the active group there was a significant increase in EPA from time 1 to time 2 (t=-9.14, $p < .0001$) and time 1 to time 3 (t=-6.33, $p < .0001$), with a slight reduction from time 2 to time 3 (t=3.70, p =0.0004). A significant decrease in the *n*-6:*n*-3 ratio was found in the active over the placebo group ($p < .001$). Post-hoc analysis showed the active group to have a significantly lower ratio at time 2 (t=7.25, $p < .0001$) and time 3 (t=4.90, $p<$ .0001). Examination of the change showed there to be no change in the *n*-6:*n*-3 ratio in the placebo group ($p > .05$) but a significant reduction in the active group from time 1 to time 2 (t=10.59, $p<$ .0001) and time 1 to time 3 (t = 8.27, $p < .0001$) with a slight increase from time 2 to time 3 (t=-

2.27, p =0.03). In both the plasma and RBCs there was no significant change in GLA or DHA (see Table 5-5).

Examination of individual change for blood levels of EPA found 2 participants in the active group who had < 50% increase from time 1 to time 2, and 1 participant in the placebo who had > 50% increase from time 1 to time 2. These participants were removed from the per-protocol analysis (for outcomes that were measured at time 1- time 2). For time 1 to time 3, 9 participants in the active group had < 50% increase indicative of non-compliance. These participants were removed for the per-protocol analysis (for outcomes measured at time 1 – time 3 and time 2 – time 3).

Table 5-5: Blood PUFA comparison by placebo and active group by the three time-points

	Time 1 M(SD)			**Time 2 M(SD)**			**Time 3 M(SD)**			**Time x Group**			
Plasma (µg/ml)	**Active (N=38)**	**Placebo (N=38)**	**T-test[a]/ MW[b]** ***p***	**Active (N=31)**	**Placebo (N=36)**	**T-test[a]/ MW[b]** ***p***	**Active (N=26)**	**Placebo (N=29)**	**T-test[a]/ MW[b]** ***p***	***p***	**Est**	**SE**	**Est 95% CIs**
EPA	19.32 (11.39)	19.69 (12.10)	0.89[a]	46.44 (24.97)	18.38 (14.72)	**<.0001[a]**	38.80 (25.73)	15.13 (9.56)	**0.0001[b]**	**< .001****	11.36	2.43	6.53 to 16.19
DHA	31.22 (19.67)	31.82 (19.38)	0.89[a]	37.16 (23.70)	27.96 (18.77)	0.06[b]	27.93 (13.34)	21.71 (11.27)	**0.07[a]**	0.18	3.02	2.23	-1.42 to 7.45
GLA	11.98 (6.32)	13.87 (9.54)	0.71[b]	11.15 (6.20)	13.45 (6.62)	0.15[a]	11.63 (5.75)	11.44 (6.14)	0.79[b]	0.20	0.89	0.69	-0.48 to 2.26
Σn-6[d]: Σn-3[e]	3.76 (1.32)	3.91 (1.51)	0.65[a]	2.06 (0.88)	4.17 (1.37)	**<.0001**	2.52 (1.46)	4.08 (1.20)	**0.0001**	**< .001****	-0.58	0.16	-0.90 to -0.25
RBC (µg/g)	**Active (N=38)**	**Placebo (N=37)**		**Active (N=30)**	**Placebo (N=35)**		**Active (N=26)**	**Placebo (N=29)**					
EPA	11.94 (5.11)	10.43 (3.83)	0.15[a]	23.47 (9.97)	9.81 (4.56)	**< .0001[b]**	20.30 (10.15)	8.82 (2.95)	**< .0001[b]**	**< .001****	4.49	0.76	2.97 to 6.01
DHA	49.37 (18.08)	44.91 (13.88)	0.43[b]	53.94 (15.77)	41.22 (12.87)	**0.0007[a]**	43.28 (9.73)	36.75 (7.41)	**0.007[a]**	0.20	2.22	1.73	-1.21 to 5.66
GLA	0.92 (0.38)	0.90 (0.33)	0.92[b]	0.74 (0.29)	0.85 (0.29)	0.12[a]	0.76 (0.29)	0.74 (0.26)	0.79[b]	0.99	0.00	0.05	-0.10 to 0.10
Σn-6[e]: Σn-3[f]	2.62 (0.59)	2.68 (0.51)	0.65[a]	1.83 (0.46)	2.78 (0.57)	**< .0001[a]**	1.94 (0.65)	2.68 (0.48)	**< .0001[a]**	**< .001****	-0.23	0.05	-0.33 to -0.13

c = Comprised of EPA, DHA and DPA, d = Comprised of DGLA, AA, Adrenic acid and docosapentaenoic acid, e = n6 hufa µg per ml/n3 hufa µg per ml, f = n6 hufa µg per g/n3 hufa µg per g, * Significant at nominal level (p ≤ .05), ** Significant after correction for multiple testing (p ≤ .006), MW – Mann-Whitney two sample rank sum test, time 1 = baseline, time 2 = 3 months, time 3=6 months

5.7.1.6 ITT analysis

The ITT analysis is shown in Table 5-6. There were no significant treatment effects on either the primary or secondary outcomes (all $p>.05$).

5.7.1.7 Sensitivity analysis

The ITT analysis after multiple imputation is in Appendix D, Table AD-9. Outcomes did not differ from the primary ITT analysis, with no significant treatment effects on either the primary or secondary outcomes (all $p>.05$).

Table 5-6: Intent to treat analysis

	Time 1			Time 2			Time 3			Treatment x time				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
Primary outcome														
SART CE	34.73 (12.84)	34.23 (12.92)	40/39	-	-	-	34.69 (14.19)	34.66 (14.09)	26/29	-1.09	1.15	-3.38 to 1.19	0.34	0.26
Secondary outcomes														
Cognition														
SART OE	20.88 (20.02)	25.36 (32.80)	40/39	-	-	-	20.31 (18.47)	18.52 (20.81)	26/29	2.87	2.60	-2.30 to 8.05	0.27	0.31
SART RTV	129.24 (62.72)	136.58 (62.42)	40/39	-	-	-	272.95 (297.43)	380.00 (410.56)	26/29	-50.75	49.11	-148.55 to 47.05	0.30	0.28
SART CV	0.40 (0.19)	0.41 (0.16)	40/39				0.79 (0.76)	1.01 (1.00)	26/29	-0.11	0.12	-0.36 to 0.13	0.36	0.26
CPT CE	3.11 (3.40)	3.21 (3.25)	36/33	-	-	-	1.64 (2.23)	2.72 (3.09)	25/29	-0.37	0.46	-1.30 to 0.55	0.42	0.22
CPT OE	1.81 (2.30)	2.00 (2.17)	36/33	-	-	-	2.08 (3.88)	1.69 (2.14)	25/29	0.25	0.41	-0.57 to 1.07	0.54	0.17
CPT RTV	137.36 (59.25)	141.84 (70.02)	36/33	-	-	-	143.45 (78.49)	120.67 (58.83)	25/29	13.10	11.70	-10.20 to 36.40	0.27	0.31
CPT CV	0.34 (0.13)	0.34 (0.14)	36/33	-	-	-	0.35 (0.23)	0.30 (0.13)	25/29	0.03	0.03	-0.03 to 0.08	0.33	0.27
Fast task MRT	759.73 (277.59)	664.19 (201.32)	41/39	-	-	-	696.38 (296.04)	586.23 (162.66)	26/28	7.54	27.93	-48.05 to 63.14	0.79	0.07
Fast task RTV	241.89 (236.40)	168.35 (124.07)	41/39	-	-	-	225.93 (318.89)	137.67 (91.40)	26/28	23.64	25.29	-26.71 to 74.00	0.35	0.26

	Time 1			Time 2			Time 3			Treatment x time				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
Fast task CV	0.29 (0.15)	0.24 (0.10)	41/39	-	-	-	0.27 (0.17)	0.22 (0.10)	26/28	0.01	0.02	-0.03 to 0.04	0.75	0.09
Fast task reward MRT	520.91 (169.96)	508.03 (119.63)	41/39	-	-	-	505.12 (158.57)	460.49 (103.18)	26/28	3.97	12.16	-20.25 to 28.19	0.75	0.09
Fast Task reward RTV	112.04 (78.52)	110.40 (66.81)	41/39	-	-	-	118.11 (151.35)	89.61 (62.09)	26/28	12.04	15.66	-19.13 to 43.22	0.44	0.21
Fast Task reward CV	0.20 (0.07)	0.20 (0.08)	41/39	-	-	-	0.21 (0.18)	0.18 (0.08)	26/28	0.01	0.02	-0.02 to 0.05	0.47	0.2
Fast task change MRT	238.81 (201.57)	156.16 (135.95)	41/39	-	-	-	191.26 (160.45)	125.74 (107.77)	26/28	-3.87	21.87	-47.40 to 39.66	0.86	0.05
ADHD Symptoms														
CW Innattention	27.10 (6.79)	27.23 (5.42)	42/39	23.16 (8.25)	23.32 (7.72)	31/37	22.60 (8.53)	24.55 (6.54)	25/29	-0.93	0.80	-2.53 to 0.67	0.25	0.32
CW Hyp/Imp	20.17 (5.45)	20.00 (6.22)	42/39	18.52 (5.64)	15.92 (6.76)	31/37	16.68 (7.30)	17.48 (5.68)	25/29	-0.43	0.63	-1.68 to 0.82	0.49	0.19
CW EL	17.79 (7.20)	18.21 (7.15)	42/39	14.94 (7.08)	15.03 (7.72)	31/37	15.08 (6.96)	14.59 (6.34)	25/29	-0.03	0.63	-1.29 to 1.22	0.96	0.01
Emotional lability														
CNS-LS	32.37 (16.74)	33.15 (16.42)	41/39	30.97 (13.21)	26.30 (15.00)	31/37	27.65 (13.95)	29.00 (16.70)	26/29	0.06	1.42	-2.78 to 2.89	0.97	0.01
ALS	24.51 (11.24)	24.10 (13.07)	41/ 39	22.29 (10.59)	20.95 (10.81)	31/37	18.73 (9.86)	22.24 (13.06)	26/29	-1.61	1.14	-3.87 to 0.66	0.16	0.39
PASAT time to quit	338.76 (135.11)	311.51 (152.46)	42/39	-	-	-	356.73 (127.05)	336.48 (136.33)	26/29	-6.87	18.29	-43.29 to 29.54	0.71	0.1
PASAT Frustration pre-task	10.93 (17.18)	14.49 (20.40)	42/39	-	-	-	11.35 (26.57)	12.55 (16.69)	26/29	-0.04	3.05	-6.11 to 6.03	0.99	0.00

	Time 1			Time 2			Time 3			Treatment x time				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
PASAT Frustration post-task	63.05 (32.02)	62.56 (33.49)	42/39	-	-	-	51.12 (35.25)	55.31 (26.19)	26/29	-2.20	3.91	-9.99 to 5.59	0.58	0.15
PASAT Irritability pre-task	13.17 (21.61)	14.49 (19.93)	42/39	-	-	-	15.23 (26.43)	17.76 (21.65)	26/29	-1.17	3.48	-8.09 to 5.75	0.74	0.09
PASAT Irritability post-task	47.02 (33.11)	49.33 (32.46)	42/39	-	-	-	41.85 (36.82)	45.69 (29.49)	26/29	-2.81	3.79	-10.36 to 4.74	0.46	0.2

Note. OE = Omission errors, CE = Commission errors, MRT = Mean Reaction Time, RTV = Reaction Time Variability, CV = Coefficient of Variation (RTV/MRT), CW = CAARS/WRAADS, time 1 = baseline, time 2 = 3 months, time 3=6 months.

5.7.1.8 Per-protocol analysis

The per-protocol analysis is shown in Table 5-7. The primary outcome showed no evidence of a treatment effect. For the secondary outcomes, there was a treatment effect at a nominal level on symptoms of inattention (p =0.04, d = 0.68). There was a trend for a reduction in emotional lability measured with the ALS (p=0.06, d=0.59), and also trends for reductions in pre-task frustration (p = 0.09, d=0.55), and post-task irritability (p=.09, d=0.57) in the active group compared to the placebo group. There was also indication of a treatment effect on pre-task irritability, mainly due to an increase in this rating in the placebo group at follow-up (p=0.10, d=0.52). Post-hoc analysis for the treatment effect on inattention showed in both the active and placebo group there was a significant reduction in symptoms of inattention from time 1 to time 2 and from time 1 to time 3. There was an indication for a reduction in the active (p =0.15) but not placebo group (p = 0.63) between time 2 and time 3 which will have led to the significant time x group interaction. This effect will have become non-significant in the post-hoc analysis due to the reduction in power when examining fewer time-points and because LS-Means (which are used to examine the contrasts) are conservative.

Table 5-7: Per-protocol analysis (for variables measured at T1-T3: participants with > 50% PUFA T1-T3 increase; for variables measured at T1, T2 and T3: participants with > 50% PUFA increase T1-T2 and T1-T3).

	Time 1			**Time 2**			**Time 3**			**Treatment x time**				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
Primary outcome														
SART CE	37.88 (11.26)	33.66 (13.72)	17/29	-	-	-	35.35 (12.15)	34.66 (14.09)	17/29	-1.76	1.35	-4.48 to 0.95	0.20	0.41
Secondary outcomes														
Cognition														
SART OE	20.65 (16.66)	23.17 (33.17)	17/29	-	-	-	19.88 (14.18)	18.52 (20.81)	17/29	1.95	3.45	-5.00 to 8.89	0.58	0.17
SART RTV	135.36 (49.06)	130.02 (62.21)	17/29	-	-	-	229.76 (220.34)	380.00 (410.56)	17/29	-77.80	55.16	-188.97 to 33.38	0.17	0.44
SART CV	0.45 (0.18)	0.39 (0.15)	17/29	-	-	-	0.71 (0.62)	1.01 (1.00)	17/29	-0.18	0.14	-0.46 to 0.10	0.20	0.41
CPT CE	4.06 (4.27)	3.17 (3.43)	16/23	-	-	-	2.13 (2.66)	2.72 (3.09)	16/29	-0.57	0.64	-1.86 to 0.71	0.37	0.29
CPT OE	1.81 (2.14)	1.48 (1.90)	16/23	-	-	-	2.81 (4.68)	1.69 (2.14)	16/29	0.38	0.50	-0.63 to 1.38	0.45	0.24
CPT RTV	137.77 (63.41)	125.51 (53.94)	16/23	-	-	-	156.12 (84.90)	120.67 (58.83)	16/29	11.56	14.58	-17.82 to 40.95	0.43	0.25
CPT CV	0.35 (0.13)	0.32 (0.14)	16/23	-	-	-	0.40 (0.26)	0.30 (0.13)	16/29	0.03	0.03	-0.04 to 0.10	0.33	0.31
Fast Task MRT	734.44 (261.69)	655.10 (190.18)	17/29	-	-	-	721.60 (357.59)	586.23 (162.66)	17/28	27.23	27.24	-27.67 to 82.12	0.32	0.31
Fast Task RTV	223.05 (157.62)	172.50 (134.09)	17/29	-	-	-	258.90 (390.80)	137.67 (91.40)	17/28	33.49	30.57	-28.12 to 95.09	0.28	0.35
Fast task CV	0.29	0.24	17/29				0.28 (0.21)	0.22 (0.10)	17/28	0.01	0.02	-0.03 to	0.78	0.09

	Time 1			Time 2			Time 3			Treatment x time				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
	(0.13)	(0.11)										0.04		
Fast Task reward MRT	552.98 (178.40)	481.80 (102.03)	17/29	-	-	-	528.69 (190.84)	460.49 (103.18)	17/28	-1.81	14.34	-30.71 to 27.10	0.90	0.04
Fast Task reward RTV	108.72 (66.77)	99.17 (57.24)	17/ 29	-	-	-	131.66 (185.29)	89.61 (62.09)	17/28	15.95	19.10	-22.54 to 54.45	0.41	0.26
Fast Task reward CV	0.19 (0.07)	0.19 (0.07)	17/29	-	-	-	0.21 (0.21)	0.18 (0.08)	17/28	0.02	0.02	-0.03 to 0.06	0.42	0.26
Fast Task change MRT	181.46 (127.87)	173.30 (130.21)	17/29	-	-	-	192.91 (187.45)	125.74 (107.77)	17/28	29.13	18.52	-8.20 to 66.46	0.12	0.49
ADHD Symptoms														
CW Inattention	28.00 (6.45)	26.86 (5.32)	30/37	23.52 (8.36)	23.19 (7.79)	29/36	22.06 (8.66)	24.55 (6.54)	16/29	-1.65	0.78	-3.20 to -0.09	0.04	0.68
CW Hyp/Imp	20.63 (5.40)	19.59 (6.10)	30/37	18.83 (5.60)	15.83 (6.83)	29/36	15.50 (6.03)	17.48 (5.68)	16/29	-0.63	0.62	-1.86 to 0.60	0.31	0.32
CW EL	19.33 (6.34)	17.95 (7.06)	30/37	15.07 (7.28)	14.86 (7.76)	29/36	13.63 (5.71)	14.59 (6.34)	16/29	-1.03	0.63	-2.30 to 0.23	0.11	0.52
Emotional lability														
CNS-LS	33.10 (16.05)	32.76 (15.84)	30/37	31.31 (13.54)	25.97 (15.08)	29/36	26.06 (10.98)	29.00 (16.70)	17/29	-0.53	1.60	-3.73 to 2.66	0.74	0.1
ALS	25.50 (10.76)	23.68 (12.42)	30/37	22.03 (10.70)	20.47 (10.57)	29/36	18.82 (10.10)	22.24 (13.06)	17/29	-2.27	1.20	-4.67 to 0.12	0.06	0.59
PASAT time to quit	367.29 (112.22)	316.93 (150.63)	17/29	-	-	-	371.94 (115.50)	336.48 (136.33)	17/29	-7.45	23.45	-54.72 to 39.81	0.75	0.1
PASAT Frustration pre-task	8.12 (12.62)	9.69 (11.67)	17/29	-	-	-	2.59 (2.50)	12.55 (16.69)	17/29	-4.20	2.39	-9.02 to 0.63	0.09	0.55
PASAT Frustration post-task	62.35 (29.09)	66.66 (31.16)	17/29	-	-	-	47.41 (30.11)	55.31 (26.19)	17/29	-1.80	4.58	-11.03 to 7.44	0.70	0.12
PASAT Irritability pre-task	9.24 (15.97)	10.24 (11.52)	17/29	-	-	-	6.35 (11.71)	17.76 (21.65)	17/29	-5.20	3.13	-11.51 to 1.11	0.10	0.52

	Time 1			Time 2			Time 3			Treatment x time				
	Active M(SD)	**Placebo M(SD)**	**N (A/P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/ P)**	**Active M(SD)**	**Placebo M(SD)**	**N (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
PASAT Irritability post-task	53.24 (33.45)	51.17 (29.00)	17/29	-	-	-	32.71 (30.57)	45.69 (29.49)	17/29	-7.52	4.16	-15.91 to 0.86	0.08	0.57

Note. OE = Omission errors, CE = Commission errors, MRT = Mean Reaction Time, RTV = Reaction Time Variability, CV = Coefficient of Variation (RTV/MRT), CW = CAARS/WRAADS, time 1 = baseline, time 2 = 3 months, time 3=6 months.

* Significant at nominal level (p≤.05)

5.7.1.9 Blinding

Midway through the study we asked participants to guess whether they thought they were taking the active or placebo medication. Blinding appeared to have been successful in the 12 participants asked in the active group, with 6 correct guesses, 5 incorrect guesses and 1 participant who did not know. In the 19 participants asked in the placebo group, 13 guessed correctly, 3 incorrectly and 3 did not know, there was no difference in the number of correct and incorrect guesses between the placebo and active group ($x^2 = 2.23$, $p = 0.14$).

5.7.1.10 Adverse events

An equal number (n=3 in each) of minor adverse events occurred in both the active and placebo group. In the active group one participant reported migraines and dropped out of the study, one participant said the supplements caused bloating, and one participant reported a 'gag' reflex when swallowing the capsules.

In the placebo group one participant reported weight gain, one participant said the supplements made them feel sick, and one participant reported feeling very anxious and unsettled after starting the trial, which may have been due to the supplements or to coming off ADHD medication for 48 hours before entering the study.

5.7.2 Discussion: Supplementation with *n*-3 PUFA in adults with ADHD will improve ADHD symptoms, emotional lability and cognition

This is, to our knowledge, the first randomised controlled trial of *n*-3 PUFA supplementation in adults with ADHD. We failed to find a significant treatment effect for our primary outcome, commission errors on the SART task.

For the secondary analyses examining changes in cognition (measured with the SART, CPT (commission (CPT only)/omission errors, RTV and CV) and the Fast Task (in the baseline and fast-incentive condition (MRT and RTV)), ADHD symptoms, and emotional lability (measured with self-rating scales and a frustration task (the PASAT)), we failed to find any treatment effects in the ITT analysis. The per-protocol analysis gave an indication that supplementation may have improved

symptoms of inattention at a moderate effect (d=0.68), albeit this was nominally significant. There was also an indication of a trend for supplementation to have reduced emotional lability, again at a moderate effect (d=~0.5) (measured with the ALS) and ratings of irritability and frustration on the PASAT with a trend for reductions in pre-task frustration and post-task irritability (d=~0.6). There was also an indication of a treatment effect on pre-task irritability (d=~0.5). The supplements were well tolerated with equal numbers (3) of mild adverse events reported in the placebo and active groups.

We failed to find a treatment effect of *n*-3 PUFA supplementation on our primary outcome, cognition, measured by performance on the SART task. We also failed to find treatment effects for our secondary cognitive performance outcomes (measured as performance on the CPT and the Fast Task). For all three variables neither the ITT nor per-protocol analysis showed any significant treatment effects. This finding is in line with our recent research where we conducted a meta-analysis examining the effect of *n*-3 PUFA supplementation on cognition in children with ADHD or who had a related-neurodevelopmental disorder and typically developing children and adults (see Chapter 2 (Cooper et al., 2015)). We failed to find evidence of a treatment effect across nine cognitive domains (which encompassed the outcomes employed in this study: inhibition (commission errors), attention (omission errors), MRT and RTV) in either the ADHD or the TD group. A treatment effect was found in the secondary analysis for only one of the domains (short-term memory) only in those with low *n*-3 PUFA status (across the TD and ADHD group). This meta-analysis did not include studies in adults with ADHD, and, to our knowledge, this is the first study to investigate *n*-3 PUFA supplementation in this population. Therefore conclusions regarding the lack of effect of *n*-3 PUFA on cognition were limited only to children with ADHD. The results here provide preliminary evidence to suggest the conclusions from our meta-analysis may also apply to adults with ADHD.

The per-protocol but not the ITT analysis indicated that supplementation may improve ADHD symptoms of inattention. Given this was found in only the per-protocol analysis and similar reductions in hyperactivity/impulsivity were not found it is possible that this is a chance finding. Despite this, an indication of improvement (in the per-protocol but not ITT) was found for emotional lability on one of the self-rating scales (the ALS), and also a trend for reductions in pre-

task frustration, and pre-and post-task irritability. Indications of improvement in two domains strengthen the possibility that these may be indications of real treatment effects. Although this cannot be deemed as sufficient evidence given that this study was underpowered to detect the small to moderate effects of *n*-3 PUFA supplementation on ADHD symptoms which have been predicted by prior meta-analyses (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013) (see limitations section for discussion of this). Despite this, these preliminary results of potential effects are in line with previous research (Bloch & Qawasmi, 2011; Cooper et al., 2016; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013) and indicate that further studies are warranted with either emotional lability or inattention as the primary outcome.

Differences in results between the per-protocol and ITT analyses are not uncommon. The per-protocol analysis includes only participants who strictly adhered to the protocol and therefore best reflects the optimum effects of *n*-3 PUFA supplementation. Whereas the ITT analysis includes all cases and is thought to best reflect practice in the real-world, where some but not all people will be treatment adherent. The ITT analysis is thought to be less prone to bias, whereas the per-protocol analysis may be more prone to bias towards a treatment effect. For example, due to selection bias, if withdrawal is due to adverse effects or non-response, this will not be included in the treatment estimate therefore inflating the effect size. Drop-outs in this study were considered to be missing at random (MAR) (this is further discussed in the limitations section), and so we therefore expect treatment effect estimates from both the ITT and per-protocol analysis to be relatively free from bias, with the per-protocol analysis reflecting the optimum effects of supplementation. However the null effects found in the ITT analysis weaken the indication of the potential treatment effects found in the per-protocol analysis.

The potential finding of a treatment effect of *n*-3 PUFA on inattention and potentially EL is further supported, given that previous research, including a study conducted by ourselves (R E Cooper et al., 2016), has suggested that *n*-3 PUFA supplementation could help improve symptoms of ADHD and potentially EL. Meta-analyses using data from randomised placebo-controlled trials have relatively consistently found a small but significant effect of *n*-3 PUFA supplementation in reducing ADHD symptoms in children with ADHD (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). We recently conducted a meta-analysis that provided suggestive evidence of

small effects of *n*-3 PUFA on reducing EL and oppositional behaviour in subgroups of children with ADHD (Chapter 3: (R E Cooper et al., 2016).

One potential explanation for the null effect of *n*-3 PUFA supplementation on cognition, and only potential indications for effects on ADHD symptoms and to a lesser extent emotional lability is that levels of *n*-3 PUFA and the *n*-6:*n*-3 PUFA ratio were similar in ADHD cases compared to controls, with ADHD cases even having slightly higher *n*-3 levels. Given results from our recent meta-analysis which found a treatment effect on short-term memory in those who were *n*-3 PUFA deficient. The seemingly 'typical' levels of *n*-3 PUFA at baseline in the ADHD sample may have rendered any effect of supplementation as relatively null.

The predominately negative results for the effect on cognition, and more suggestive evidence for an effect on the behavioural domains of inattention and, potentially, emotional lability, is in line with the suggestion that ADHD treatment may be more effective for the behavioural symptoms (such as inattention and emotional lability) than cognitive performance measures (Coghill et al., 2014). For example, smaller treatment effects for stimulant medication have been found for cognitive performance (d=~0.2-0.6) (Coghill et al., 2014) than for ADHD symptoms (d=~0.8-1.0) (Banaschewski et al., 2006; Faraone & Buitelaar, 2010). This is also illustrated by the findings from this trial and meta-analyses with a generally null effect of *n*-3 PUFA on cognition (Cooper et al., 2015), but suggestive evidence for an effect on emotional lability (R E Cooper et al., 2016) and established evidence for an effect on ADHD symptoms in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). It has also been suggested that different neural mechanisms may be responsible for change in cognitive performance and change in behavioural symptoms (Coghill et al., 2007); following the observation that during the clinical response to methylphenidate there is a dissociation of the treatment effects on ADHD symptoms and cognitive performance in children and adolescents with ADHD (Bédard et al., 2014; Coghill et al., 2007; K. P. Schulz et al., 2014).

Potential effects of *n*-3 PUFA on the behavioural symptoms of ADHD may be due to reduced inflammation and alterations in neurotransmission; mechanisms implicated in the pathophysiology of ADHD. Through production of eicosanoids (lipid mediators that are involved in a wide array of

physiological functions including inflammation) (Janssen & Kiliaan, 2014; Schmitz & Ecker, 2008; Simopoulos, 2011), *n*-3 PUFAs may limit neuroinflammation whereas *n*-6 PUFAs may promote inflammation (Gow & Hibbeln, 2014; Sinn, Milte, & Howe, 2010; Janssen & Kiliaan, 2014; Simopoulos, 2002). Alongside ADHD, inflammation has been linked to a number of other mental health problems including depression, schizophrenia and bipolar disorder (Dean, 2011; Raison & Miller, 2013; Strickland, 2014). Through altered cellular communication, *n*-3 PUFA deficiencies may lead to altered neurotransmission particularly for dopamine and serotonin (Assisi et al., 2006; Chalon, 2006; Haag, 2003; Young & Conquer, 2005). Disrupted dopamine signalling is a well-established theory of ADHD (see Section 1.1.9.4) with serotonergic genes having also been associated with the disorder (Faraone et al., 2005; Gizer et al., 2009).

There are several important limitations to be considered before conclusions can be drawn. This study was limited by a high drop-out rate, with a total drop-out of 16% by time 2 and 32% by time 3. This high drop-out rate will have considerably reduced power in the analysis. Although more drop-outs occurred in the active than placebo group (16 vs 10) this difference was not statistically significant and reasons for drop-out appeared to be similar across both groups. Furthermore, ADHD symptom severity did not differ between drop-outs and non-drop-outs, providing some evidence to suggest that those who remained in the trial were no less severe in their symptoms than those who dropped-out. We therefore considered data to be missing at random (MAR), and used a linear model to assess outcomes. Linear models are said to reliably yield unbiased estimates of treatment effect (Bell, Kenward, Fairclough, & Horton, 2013; Molenberghs et al., 2004). Therefore we feel that despite the high drop-out rate, the results presented here may be relatively free from bias.

The main reason for drop-outs was due to loss of contact. This is a problem that could be considered inherent to the disorganised nature of the ADHD condition. Appointment cancellations and no-shows were a common part of the study, increasing the difficulty of the research process. It is estimated that around 30-40% of participants cancelled or re-arranged their appointments, often at short notice. For some of these participants, multiple cancellations were made before we lost contact. It is interesting to note that a 6-month placebo-controlled RCT examining the effects of stimulant medication in adults with ADHD also had a high drop-out rate with a 38% completion

rate in the active, and 50% completion rate in the placebo group. The main reason for drop-outs, as in the current study, was loss of patient contact (Adler et al., 2008).

Another reason for drop-outs may have been the longer length of the trial (6 months) during which time patients may have lost interest or found it too demanding to take the supplements every day. In line with this, although increases in blood *n*-3 PUFA levels were found in the active group from baseline to the final visit, this was primarily driven by increase from baseline to time 2 (3 months). Slight decreases in blood PUFA levels were found between 3 months and 6 months. This suggests participants were less likely to adhere to taking the supplements in the second half of the study. Furthermore, in the per-protocol analysis 9 participants from the active group were excluded whose *n*-3 PUFA blood levels did not increase from baseline to the final visit. When examining time 1 to time 2 changes, only 2 participants from the active group were excluded. Future studies in this population may consider a trial of shorter length (3-4 months).

It is also possible that drop-outs may have occurred because the baseline testing sessions were long and cognitively demanding, which may have caused unwillingness for participants to return. One participant could not tolerate the EEG session, and a number of other participants expressed that they found this session difficult to complete. It is of note that the second trial conducted in this thesis (Chapter 6) employed shorter testing sessions (~3 hours versus ~5 hours) and a shorter follow-up period (6-weeks vs 6-months) than this study and had a significantly lower drop-out rate (17% versus 32%).

From the sub-sample of those that were asked to guess which medication they were taking, blinding appeared to have been more successful in the active than placebo group (although this difference was not statistically significant). Given that a larger number of participants (12 versus 19) were asked in the placebo than the active group, it is difficult to draw a firm conclusion. Failure of the blinding in the placebo group could potentially have led to an underestimation of the treatment effect. Although greater bias as a result of failure of the blind is often observed more for subjective outcomes, therefore rating scale measures of EL and ADHD symptoms may have been affected more than our primary outcome of cognition (Wood et al., 2008).

The main limitation to this study is that we were underpowered to detect the small to moderate effects estimated from previous meta-analyses. Meta-analysis examining the effect of *n*-3 PUFA supplementation in children with ADHD have estimated the effect to be at around d=0.3 (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). At this effect a sample size of 352 participants would be required to detect an effect at a nominal level of significance ($\alpha = 0.05$). However, given that this was a pilot study, the main aim was to evaluate the feasibility of the study, and potential trends and effect sizes, which would indicate positive effects of *n*-3 PUFA supplementation in order to guide a future, larger study. This has been achieved, as the per-protocol analysis indicated potential treatment effects on inattention and emotional lability which is in line with previous meta-analysis (Bloch & Qawasmi, 2011; Cooper et al., 2016; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). Observations from running the experiment can also be used for future trials such as the need for a shorter follow-up period and testing sessions in order to attempt to limit drop-out rate.

In conclusion, this pilot study has found no evidence for an effect of *n*-3 PUFA supplementation on cognition, and only an indication of possible improvements in inattention, and to a lesser extent emotional lability. These conclusions are limited by the high drop-out rate and low power in this study. Future studies should investigate treatment effects in a larger sample size, with a shorter follow-up period (~ 3-4 months) and shorter, less cognitively demanding testing sessions (~ 2 hours total).

5.8 Overall conclusion

This chapter has found a sample of adults with ADHD to have more severe symptoms of inattention, hyperactivity/impulsivity, emotional lability, and impaired cognitive performance, compared to a control group. This supports the DSM-5 diagnostic criteria which alongside typical ADHD symptoms, lists emotional lability and cognitive impairment as associated features of ADHD; demonstrating that the sample here is characteristic of ADHD clinical populations and the data is therefore generalisable to other ADHD samples. Alongside this we have found evidence supporting the developmental stability of sensitivity to reward and presentation rate on cognitive (reaction time) performance in ADHD. We have also provided objective evidence for emotional overreactivity in adults with ADHD and proposed that the PASAT-C could be used as an objective measure of

frustration in this group. We then did not find any evidence that adults with ADHD had deficiencies in *n*-3 PUFA blood levels, even finding evidence contrary to this hypothesis with slightly higher levels of the *n*-3 PUFA DPA. Following this we found no evidence that supplementation with *n*-3 PUFA effects cognition with marginal evidence for effects on inattention and to a lesser extent emotional lability. A larger trial would be needed in order to gain a more conclusive picture. As a whole we have found only very marginal evidence to support the efficacy of *n*-3 PUFA as an alternative treatment in adults with ADHD.

5.9

Chapter 5 interim summaryChapter 5 addressed the second aim of this thesis (see Section 1.4), to examine the effect of *n*-3 PUFA supplementation in adults with ADHD. In agreement with the first hypothesis suggested in this aim, at baseline the ADHD group compared to controls had more severe symptoms of ADHD, higher EL and impaired cognition. Against the second hypothesis, no differences in *n*-3 PUFA blood levels were found that might have indicated a role for dietary deficiency. Going only slightly in support of the third hypothesis, no evidence for an effect of *n*-3 PUFA on cognition was found with very limited evidence for an effect of *n*-3 PUFA on inattention and potentially EL. These results, combined with evidence from Chapters 2 and 3 (see sections 2.11 and 3.6) and prior studies (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), have increased our understanding of the effect of *n*-3 PUFA supplementation in children and adults with ADHD. We know that in children with ADHD, *n*-3 PUFA supplementation appears to have a small effect on improving symptoms of ADHD with suggestive evidence for a small effect on reducing symptoms of EL and oppositional behaviour. Effects on cognition appear to be negligible in both the general population and children with ADHD (aside from a potential small effect in those who are *n*-3 PUFA deficient). In line with this pattern of larger treatment effects on behavioural symptoms than cognition, this chapter has suggested, in adults with ADHD, there is no indication for an effect of *n*-3 PUFA supplementation on cognitive performance, with limited evidence for an effect on inattention and potentially EL. We have also shown that whilst case/control differences in *n*-3 PUFA levels may be present in children (Hawkey & Nigg, 2014), this may not be the case in adulthood. We have finally given evidence to build on the presence of cognitive impairments (including evidence of reward sensitivity) and increased symptoms of EL (including objective evidence of problems with emotion regulation/emotional overreactivity) in adults with ADHD.

Given the weak evidence that we have found in this chapter for *n*-3 PUFA as an alternative treatment for adults with ADHD, it is important that other treatments are investigated. In line with this and aim 3 of this thesis (see Section 1.4) the following chapter, using a similar RCT design, investigates the cannabinoid medication, Sativex Oromucosal Spray, as an alternative treatment for adults with ADHD. In line with previous chapters, treatment effects on cognition, ADHD symptoms and EL are investigated.

Chapter 6: The effects of Sativex on neurocognitive and behavioural function in adults with attention-deficit/hyperactivity disorder: The EMA-C Study (Experimental Medicine in ADHD – Cannabinoids)

6.1 Abstract

A number of adults with ADHD appear to 'self-medicate' with cannabis, sometimes reporting a preference for cannabis over their stimulant medication. Treatment with stimulants is not always effective or tolerated, and long term treatment may be a concern in some cases; investigation of alternative treatments is therefore vital. Given this, here we report, to our knowledge, the first randomised placebo-controlled trial examining the effect of cannabinoids, using Sativex Oromucosal Spray, on ADHD associated cognitive impairments and symptoms in 30 adults with ADHD. A significant treatment effect was found for improvements in hyperactivity/impulsivity, with non-significant trends indicating potential effects in inattention and to a lesser extent cognitive performance. Adults with ADHD may represent a subgroup of individuals that gain cognitive enhancement and reduction of impairing symptoms from cannabinoid treatments. These data suggest that Sativex has potential as an alternative treatment for adults with ADHD. Future research is required in order to gain a more conclusive picture.

6.2 Introduction

Attention deficit/hyperactivity disorder (ADHD) affects around 5% of children (Polanczyk et al., 2007, 2014), continuing into adulthood in around two-thirds of cases with a prevalence of around 2.5% (Faraone et al., 2006). Adult ADHD is associated with significant clinical and psychosocial impairment with the presence of a comorbid disorder in around 75% of cases (Kooij et al., 2010). Substance use disorder (SUD) is a common comorbidity of ADHD (Arias et al., 2008; Biederman et al., 1995) with the most common drugs of abuse appearing to be stimulants and cannabis (Asherson, Evans, Kuntsi, & Young, 2015; Joseph Biederman et al., 1995; Dennis et al., 2004; Gudjonsson et al., 2012; Huntley et al., 2012). One theory posited to explain the increased risk of SUD in ADHD is that of self-medication.

Several key points underpin the self-medication hypothesis for substance abuse in ADHD. High rates of substance abuse have been found in those with undiagnosed ADHD compared to those without ADHD symptoms (Gudjonsson et al., 2012; Young & Thome, 2011), and it has been suggested that this may be related to self-treatment (Bolea-Alamañac et al., 2014). There might be improved function with drug use. For example, different motivations behind drug use have been found, with ADHD cases more likely to use drugs to improve their mood and sleep whereas those without ADHD for 'getting high' (Horner & Scheibe, 1997; Wilens, 2004). Taking into account that stimulant medication is the recommended first line treatment in ADHD and, alongside cannabis, stimulants are one of the most common classes of drugs of abuse (Biederman et al., 1995; Dennis et al., 2004; Gudjonsson et al., 2012; Huntley et al., 2012), this may indicate that those with ADHD are more likely to use drugs that have been found to alleviate symptoms of ADHD. This is further supported by the potential overlap in neurotransmitter action between stimulant medication (Leonard et al., 2004; Volkow et al., 2002) and cannabis (Bossong et al., 2009, 2015; Kuepper et al., 2013), both of which may act as dopamine agonists (see Chapter 1, Section 1.3.3 for further discussion).

In line with the self-medication hypothesis, a number of individuals with ADHD report a preference for cannabis over stimulant medication in the treatment of their symptoms. Some patients have reported an improvement in behavioural symptoms when using cannabis (Asherson, personal communication). They report feeling reduced emotional lability, restlessness and distractibility, with improved concentration, ability to sustain focus and sleep. Sativex Oromucosal Spray (GW Pharma Ltd, Salisbury, UK), a standardised cannabinoid medication, was recently prescribed for a one month period to a patient at the Maudsley ADHD Clinic, since the patient found stimulants to be ineffective and even to exacerbate his ADHD symptoms. Treatment with Sativex resulted in improved control of ADHD symptoms, behaviour and cognitive function, reported by the patient and corroborated by his mother. Although impairments in, for example, cognition (e.g. increased omission[8] and commission[9] errors and mean reaction times) have been found in healthy

[8] Where a participant fails to respond where a response is required during a cognitive task
[9] Where a participant responds when a response is not required during a cognitive task

recreational cannabis users following acute administration of Δ9-THC[10] (McDonald, Schleifer, Richards, & de Wit, 2003; Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009; Ramaekers et al., 2006), this is not consistent with the subjective accounts of patients with ADHD who may potentially represent a subgroup that respond more positively to cannabinoids.

Research into alternative treatments in ADHD is important. Stimulant medication, the first line treatment for adults with ADHD, can have a number of adverse-effects including sleeplessness, loss of appetite, and mood effects (Faraone et al., 2015; Leonard et al., 2004; Sangal et al., 2006). In other cases they have been associated with cardiovascular effects, growth suppression, and the development of psychosis or other psychiatric conditions (FDA, 2006). Furthermore partial response is common (Bolea-Alamañac et al., 2014) and clinical trials indicate that not all cases of ADHD respond to stimulant medications.

6.3 Objectives

Despite the wide use of cannabis in adults with ADHD, positive subjective patient reports, and beneficial effects found from the treatment of one patient with Sativex (Asherson, personal communication), to our knowledge the effect of a cannabinoid based medication on cognitive and behavioural function in ADHD has yet to be tested. In line with this the main objective of this study is to provide preliminary data on the relationship of short-term cannabinoid-based treatment (with Sativex Oromucosal Spray) on cognitive and behavioural measures in adults with ADHD. The following hypotheses were tested: 1) That treatment with Sativex would improve cognitive performance and 2) That treatment with Sativex would improve symptoms of ADHD and emotional lability.

[10] One of the main psychoactive compounds in cannabis (see Chapter 1, Section 1.3.1)

6.4 Methods

6.4.1 Design

The study was a 6 week[11] double-blind, placebo controlled, parallel group pilot study with balanced randomisation in 30 adults with ADHD. Participants were divided into 15 who received the active Sativex treatment and 15 who received the placebo. Participants underwent a 2 week titration period before continuing at their optimum dosage for 4 weeks. Outcomes were assessed at baseline and 6 weeks (day 42) (see Figure 6-1). The RCT analysis was presented in accordance with guidance from the CONSORT Statement (Consolidated Standards of Reporting Trials) (Schulz, Altman, Moher, & Group, 2010).

6.4.2 Changes to trial design

The original protocol included outcomes at 3 time-points (baseline (time 1), 2 weeks (time 2) and 6 weeks (time 3)). Participants attended the Social, Genetic and Developmental Psychiatry (SGDP) Centre for their time 1 and time 3 assessments. For the time 2 assessment, participants were provided with questionnaires and a stamped addressed envelope and asked to complete and post these back to us after 2weeks. However only 16/30 participants did this. Furthermore it became clear that due to a large amount of individual variation during the titration phase of the protocol, a 2 week period was too short-a-time to assess medication efficacy. Therefore the time 2 assessment was dropped from the analysis.

[11] 6 weeks was chosen in line with the duration of previous RCTs of Sativex in patients with Multiple Sclerosis; or who had neuropathic pain, which found efficacy of Sativex after 5-6 weeks (Collin et al., 2007; Nurmikko et al., 2007) (there are no studies of Sativex in adults with ADHD therefore trial duration could not be based on RCTs in this population).

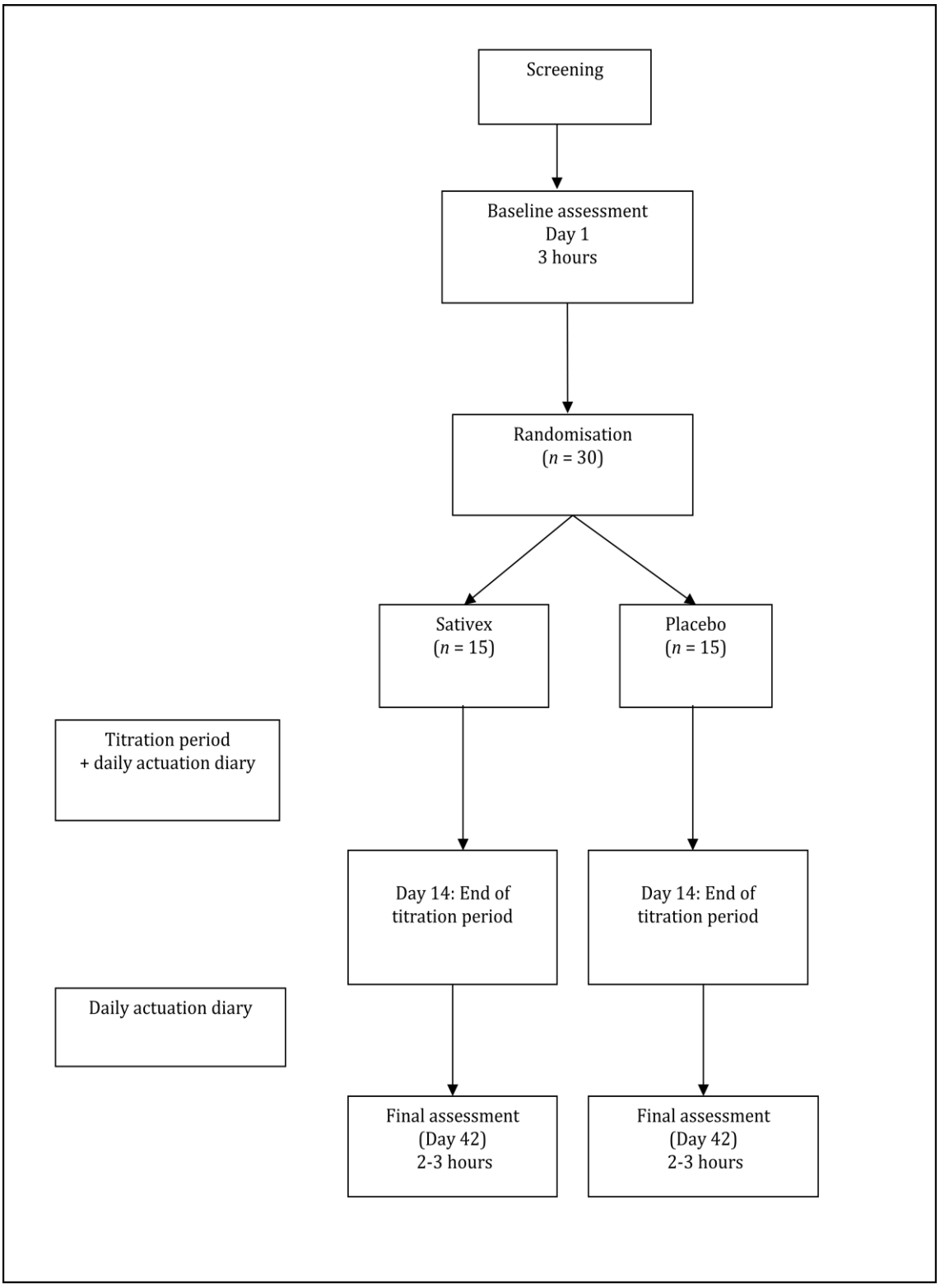


Figure 6-1: EMA-C Study design.

6.4.3 Inclusion/exclusion criteria

The inclusion/exclusion criteria is reported in Chapter 4, Section 4.3.2.1.

6.4.4 Study settings, funding and ethical approval

Study settings, funding and ethical approval are reported in Chapter 4, Section 4.3.1.1.

6.4.5 Interventions

The active and placebo medication were stored and dispensed by the Maudsley Hospital pharmacy (part of The South London and Maudsley NHS Foundation Trust (SLaM)). Both the active and placebo medications were flavoured with peppermint and were identical in appearance and method of administration (oromucosal sprays). Treatments were prescribed by one of two qualified medical doctors experienced in the diagnosis and treatment of ADHD (Prof Philip Asherson or Dr Céline Ryckaert). Participants were asked to begin the medication the morning after the baseline visit.

Active treatment

Sativex Oromucosal Spray (GW Pharma Ltd, Salisbury. UK). Each 100 microlitre spray contains the cannabinoids: 2.7 mg delta-9-tetrahydrocannabinol (Δ9-THC) and 2.5 mg cannabidiol (CBD). Sativex has similar properties to cannabis resin ('hash') which is commonly used recreationally. Δ9-THC and CBD are thought to have opposite effects with Δ9-THC associated with the psychoactive effects of cannabis (i.e. euphoria, alteration of perception, psychotic symptoms (in some individuals)) and CBD, the anxiolytic effects (Englund et al., 2012). CBD has been found to offset the psychoactive effects of Δ9-THC (Englund et al., 2012) (See also Chapter 1, Section 1.3.1).

Placebo treatment

The placebo treatment was an oromucosal spray containing ethanol, propylene glycol (50:50) excipients, with peppermint oil (0.05%) flavouring, and colourings.

Both the active and placebo treatment were identical in appearance and flavour (peppermint). They were stored in the pharmacy of the Maudsley Hospital and were prescribed by either Prof Philip Asherson (MBBS) or Dr Céline Ryckaert.

6.4.5.1 Titration period and dosing

All participants in both arms of the study underwent a two week titration period, after which they continued at the final optimal dose. At the end of the baseline assessment they received a 'dosing diary'. The diary contained titration and dosing instructions and asked the participant to record the number of sprays taken each day. The two week titration period was conducted according to a standardised dosing schedule (advised by GW Pharma) whereby treatment was increased daily (see Appendix E, Supplement AE-1). The maximum dose for the study was 14 sprays per day. During the titration and remaining 4-week period safety monitoring and evaluation of the effects of the spray were carried out on days 4, 8, 12, 14 and 28. On these days participants were called by a researcher (myself, supervised by Prof Asherson) and asked to complete a standard side-effect rating scale, the 18-item Conners' Adult ADHD Rating Scale (Conners et al., 1999), and general questions regarding their current dosage and whether they were finding any effects or adverse effects from the treatment (see Appendix E, Supplement AE-2 for titration documents). If in the opinion of the participant or myself, there were minor adverse effects that could be exacerbated at a higher dose, the participant was advised to either stay at the dose they were at or reduce to a lower dose. Titration upwards was also stopped if all ADHD symptoms were scored as negligible or absent (score of 0 or 1 on all items of the CAARS). Participants were advised to spread the doses out throughout the day as best suited them, taking into account any minor adverse events, symptom score on the CAARS, and the length of time the effect from each spray lasted. On day 14 it was decided between the participant and myself as to the optimal dose for them to continue for the remaining four weeks of the trial. Those who did not report effects from the spray were advised to titrate up to the maximum dosage and then continue at a dose they felt they could manage to take for the remainder of the study. Any queries or concerns were discussed with Prof Asherson.

6.4.5.2 Alteration to titration period and dosing

After the first 2-3 participants had begun the trial it became clear that the titration schedule advised by GW Pharma was too high for adults with ADHD. This is most likely because this recommended schedule was advised for symptom relief in Multiple Sclerosis (Sativex is licensed for treatment of MS). It was found that participants in this trial required around 4-8 sprays (mean number of sprays used = 4.73 (range = 1-13)) instead of 14. In a few cases patients double dosed and found this was associated with minor adverse experiences. In addition, the medication effects were reported as lasting around 3-4 hours in most cases. We therefore altered the protocol by informing patients at the end of the baseline session, that for some people the titration schedule was too high, and that if they found a beneficial effect from the medication they should not take another dose until that effect had started to wear off. The close monitoring of participants during the titration period (described above) ensured participants remained aware of this and that the titration process was individualised to the reported response from each patient.

6.4.6 Outcomes

6.4.6.1 Baseline only measures

- The MINI 6.0 (Mini International Neuropsychiatric Interview) diagnostic interview was used to screen for comorbid disorders (Lecrubier et al., 1997).
- Two subtests (vocabulary and matrix reasoning) of The Weschler Abbreviated Scale of Intelligence – II (Weschler, 2005) were used to measure IQ.
- Socio-economic status (SES) was assessed by collecting information on participants' level of education, occupation and income.
- Information regarding the participants' medication, ADHD diagnostic status and cannabis use was also collected.

6.4.6.2 Primary outcome

The Quantitative Behavioural Test (Qb Test) (Bijlenga et al., 2015; Iberstadt, 2012): The Qb Test is a 20 minute, unconditional identical pairs test. During the test four different stimuli (red and blue squares; red and blue circles) are presented in a random order for 200 ms with a 2- second inter-stimulus interval. If a stimulus matches the previous stimulus it is a target, otherwise it is a non-target. In total 600 stimuli are shown at a 25% target ratio. The participant is asked to respond to a

target by pressing a clicker with the thumb of the dominant hand and to inhibit responses to non-targets. During the test head movements are measured by means of a high-resolution motion tracking system that consists of an infra-red camera and a reflector attached to the participant's headband. Outcome measures are calculated per test quartile, each representing five minutes of the test duration. The first quartile is not taken into account in the outcome measures because it is least indicative for ADHD. Three cardinal outcomes are computed as Q-scores (z-scores after comparison of the participant's raw scores to the normative data matched for age and sex): QbInattention (containing omission errors, reaction time and reaction time variation), QbActivity (containing time active, distance, area, and micro-events), and QbImpulsivity (containing commission errors and normalized commission errors). The primary outcome was the mean of these three cardinal outcomes (this measure has been found previously to be sensitive to medication effects in adults with ADHD (Bijlenga et al., 2015)), with post-hoc analyses conducted on each outcome separately.

6.4.6.3 Secondary outcomes

ADHD symptoms:

- ***Conners' Adult ADHD Rating Scales (CAARS)*** (Conners et al., 1999) ***and Wender-Reimher Adult Attention Deficit Disorder Scale (WRAADS)*** (Wender, 1995) ***combined (CAARS/WRAADS: investigator rated):*** Assessed ADHD symptom severity. Total scores for each category of inattention, hyperactivity/impulsivity, and emotional lability were used in the analysis.

Emotional lability:

- ***Emotional lability: :*** The Centre for Neurologic Study Lability Scale (CNS-LS) (Moore et al., 1997) and Affective Lability Scale-Short Form (ALS-SF) (Oliver & Simons, 2004) measured emotional lability . Total score from each was used in the analysis.

Cognition:

- **Sustained Attention to Response Task (SART)** (O'Connell et al., 2009)**:** The SART is a computerised go/no go task measuring both response inhibition and sustained attention. It consists of nine digits presented in random order on a computer monitor. Participants are instructed to withhold responses to the digit 3 (no-go trial) but to respond with a button press after all other digits (go trial). Participants completed the SART over three blocks, each lasting approximately 5 minutes. Individual blocks consisted of 225 digits, with each digit presented 25 times. Stimuli were

presented in five digit sizes (font size 100, 120, 140, 160 and 180 in Arial text), subtending approximately 1.7°, 2.1°, 2.4° and 2.7°, respectively in the vertical plane. Digits were presented .31° above a central white fixation cross on a grey background for 150 ms followed by an inter-stimulus interval of 1000ms. Measures recorded were as follows: commission (where the participant responds where a response is not required) and omission (where a participant fails to respond when a response is required) errors were added across the three trials. For reaction time variability (RTV) and the coefficient of variation (CV) (SDRT/MRT) the average was computed across the three trials (mean reaction time (MRT) was not used as an outcome variable as our previous study (Chapter 5) found no difference in SART MRT between ADHD cases and controls).

Functional impairment

- **The Weiss Functional Impairment Rating Scale Self Report (WFIRS-S)** (Weiss, 2007): Covers various aspects of function including social and family relationships, self-concept and work.

6.4.7 Procedure

All participants were sent a letter by post, confirming their agreed appointment time and date. For the baseline (day 1) assessment, participants were reminded to stop taking their ADHD stimulant medication for 1 week before and for the 6-week duration of the study (7 weeks in total). For the final (day 42) assessment participants were asked to bear in mind that they would be asked to take a dose of their study medication as soon as they arrived at the appointment. For the baseline and final assessment, participants were asked to refrain from consuming alcohol on the day of the study session or the day before. Appointment reminders were given the day before the first and the final research appointment.

At the baseline visit participants read the study information sheet and completed the consent form. They then completed the investigator-rated CAARS/WRAADS, general information sheet (e.g. SES and cannabis use) and questionnaires, following this the cognitive testing session (QB Test and the SART) was carried out and lastly the IQ test and the MINI. At the end of the baseline visit participants received: a 7-week supply of either the placebo or active medication, a study diary (which contained instructions for the titration period and remaining four weeks of the study and

where participants were asked to record the number of sprays they used per day) and a confirmation letter for their final visit.

At the final (day 42) visit, participants completed an identical testing session to the baseline visit although they did not complete the IQ test or the MINI. At this final assessment participants were asked to take one dose of the medication when they first arrived, they then began the cognitive testing session 1 hour later. Therefore at time 1 and time 3, if the questionnaires took longer than one hour to complete, they would be filled out at the end of the testing session. The assessments and timings for the baseline and final assessment are detailed in Chapter 4, Table 4-2. The study design is detailed in Figure 6-1. All participants were compensated for their travel.

6.4.8 Sample size

As this was an initial pilot study, examining feasibility and trends in drug effects, formal power calculations were not required. Furthermore, as there were no prior estimates of the effects of Sativex (or indeed any cannabinoid medication) in adults with ADHD, initial estimates of an effect size for power calculations were not available. We were also conscious of the low cost-benefit ratio for participants, since this study did not meet the criteria for a clinical trial of a CTIMP under MHRA regulations (following a formal review of the protocol) and we were not able to recommend Sativex as a treatment for ADHD, even for participants who found the medication to be effective. A relatively small study aimed at large clinical effects therefore seemed warranted for such an early investigation of the effects of Sativex in adults with ADHD.

With a sample size of 30 participants and 80% power we are able to detect large effects at both nominal levels of significance ($d = 1.06$, $\alpha = 0.05$) and at trend level ($d = 0.95$, $\alpha = 0.09$). This seemed a reasonable target effect size for an initial exploratory study on the basis that patients who self-medicate with cannabis report marked improvements in ADHD symptoms, similar to that when using stimulant medication. The effect size around d=1.0 is comparable to that reported in some, but not all, meta-analyses (range $d \sim 0.6$-1.0) for stimulant medication on ADHD symptoms (Faraone & Buitelaar, 2010; Faraone & Glatt, 2010; Faraone et al., 2004; Mészáros et al., 2009).

6.4.9 Randomisation

6.4.10 Sequence generation

The randomisation list was produced by an independent statistician who produced a treatment allocation schedule to two equal-sized blocks (treatment and placebo) at random via a random number generator in the R statistical package (using the sample.int() function).

6.4.11 Allocation concealment

The randomisation list was sent to the hospital pharmacy where the medication was labelled and blinded before being dispensed. Each participant ID (1-30) corresponded to an equivalent medication number (1-30). Randomisation lists were held by the SLaM pharmacy and the independent statistician. The allocation sequence was concealed from the researchers in sequentially numbered, opaque, sealed envelopes which were kept in a locked drawer in the SGDP Centre. Neither the statistician who produced the randomisation schedule nor the pharmacist who labelled and blinded the medication were involved in any other aspect of the study. Emergency unblinding was possible with 24 hour access to a mobile phone number with a member of the research team and access to a trained clinician (Professor Asherson or Dr Céline Ryckaert).

6.4.12 Blinding

Investigators and participants were all blind to treatment allocation. Post-intervention participants were asked which group they thought they were allocated to. These estimates were used to assess the maintenance of blinding. The study was unblinded only after all data had been collected and cleaned (the final cleaned anonymised datafile was stored and sent to colleagues unrelated to the trial prior to unblinding).

6.4.13 Statistical methods

Baseline comparisons were carried out using STATA (StataCorp, 2009). For categorical data, either a Chi-squared or Fisher's Exact (if expected frequencies were 5 or less) test were used. For continuous data either a T-test or Mann-Whitney two sample rank sum test (if data were non-normal) were used. Normality for baseline comparisons was assessed by conducting the skewness

and kurtosis tests for normality, examination of the value of skew (-1 to 1 considered normal) and inspection of the histogram.

The Intent-to-treat (ITT) and per-protocol analyses were conducted in SAS®. The ITT analysis included every participant who was randomised to the trial, regardless of protocol deviations, compliance and withdrawal (Lewis & Machin, 1993), and was considered the primary analysis. Per-protocol analysis included only those patients who adhered to the protocol (Lewis & Machin, 1993), and was considered the secondary analysis. The covariance structure of the data was examined and no discernible pattern was found: therefore treatment effects were estimated (in both the per-protocol and ITT analysis) using a repeated measures linear model (with fixed effects: Group, Time and the interaction of these two terms) with an unstructured covariance matrix (Kincaid, 2005) (the procedure MIXED was used). A significant group x time interaction indicated a treatment effect. Linear models are robust to deviations from normality (Hamer & Simpson, 2000) and modelling the covariance structure of repeated measures is considered an effective method to handle missing data without the need for multiple imputation (MI) (Gadbury et al., 2003).

To examine whether or not the missing data influences the linear model estimates, multiple imputation (MI) was implemented (using the PROC MI procedure in SAS) as a sensitivity analysis, and performed using the ITT populations. Two types of MI analyses were performed: 1) the first assumed that missing data were missing at random (MAR) and 2) the second method of imputation assumed that the missing data might relate to the value and thus is missing not at random (MNAR) (Allison, 2012; Twisk et al., 2013; White, Carpenter, & Horton, 2012; Yuan, 2014). Given that data was missing in both monotone and arbitrary patterns, two separate MIs under the MAR assumption were conducted; the first used a monotone simulation (which imputed only data that were missing at follow-up), and the second used an arbitrary simulation with the Fully Conditional Specification (FCS) method (which imputed data at baseline and follow-up). Both the monotone and FCS MIs used all available observations to derive the imputation model. Imputation under the MNAR assumption used a control-based pattern mixture model which assumed data to be missing in a monotone pattern (and imputed values only at follow-up). Under this model it was assumed that individuals who dropped out of the treatment group would no longer be receiving treatment and would have outcomes similar to the placebo group. Therefore imputation in both the placebo

and active group was based only on the placebo group (Yuan, 2014). A nominal level of significance was set at $p < .05$ and at $p < 0.004$ after correction for multiple testing (Bonferroni correction for 14 statistical tests). Given this was a pilot study we also examined trends ($p < .09$) and indications ($p< .20$) of treatment effect. Effect sizes were classified according to Cohen's *d* (0.2 = small, 0.5 = medium, 0.8 = large) (Cohen, 1988).

6.5 Results

6.5.1 Participant flow, losses and exclusions

Participant flow through the study is shown in Figure 6.1, and detailed reasons for exclusions in Chapter 4 (Figure 4-2 and Appendix C, Table AC-6). Of the 233 potential participants that were screened 203 were excluded due to: not meeting inclusion criteria (n=99), declined to participate (n=18), other reasons which included non-response to being contacted about the trial (n=56), not sufficient information to screen (n=25), and recruited for the study and then declined before being booked to begin (n=5). For those who failed to meet inclusion criteria the main reasons were: that they did not have enough ADHD symptoms (n=19), presence of a co-occurring mental health condition (n=15), unwilling or unable to come off current ADHD medication (n=12), current psychoactive medication (other than stimulants) (n=11), history of current or past substance abuse/dependence (n=9), physical health problems (n=7), and unwillingness to take a cannabinoid medication (n=5).

The participants were 30 adults with ADHD (11 female, 19 male, mean age = 37.9 years (SD = 11.46), research diagnosis N=7, clinical diagnosis N=22, childhood diagnosis and adult research diagnosis N=1). The majority of participants met criteria for the combined type presentation of ADHD (N=26) with only a small number with the inattentive type presentation (N=4). Of the 23 with a prior clinical diagnosis, five had been diagnosed in childhood and 18 in adulthood. The participants were randomised in a 1:1 ratio (15 active, 15 placebo).

Of the 30 participants, complete follow-up data was not obtained from two participants in the placebo group. One participant experienced an adverse event (see section 6.5.10 'Adverse events'). Contact was lost with the second participant. Partial follow-up data was obtained from two other participants who failed to return for follow-up, however, both completed the CAARS/WRAADS,

CNS-LS, and ALS-SF over the telephone. Of these participants, one failed to attend due to a head injury which meant they stopped taking the study medication. The second failed to attend their final appointment for logistical reasons. In the active group, one participant experienced an adverse event two weeks into the trial and stopped taking the study medication, but attended their follow-up session as scheduled (see section 6.5.10 'Adverse events'). At baseline one participant in the active and one in the placebo group could not tolerate the Qb Test. Due to technical difficulties two participants in the placebo group (one at baseline, one at follow-up) could not complete the SART.

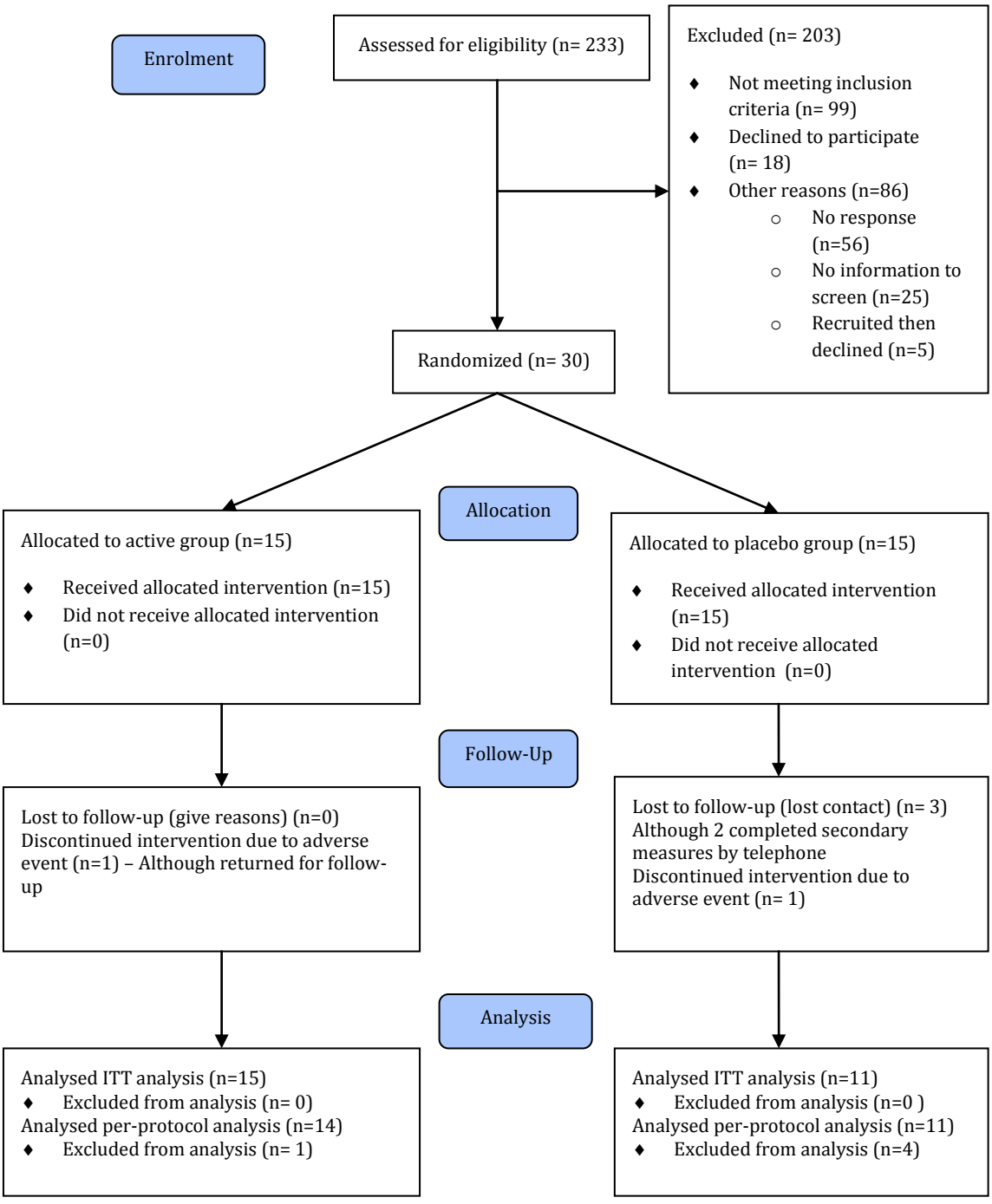


Figure 6-2: CONSORT flow diagram for EMA-C Study

6.5.2 Recruitment

Eligible participants were recruited from July 2014 to May 2015. Participants came to the SGDP Centre for baseline (time 1) and 6 week (time 3) testing sessions.

6.5.3 Baseline data

The baseline demographics for the placebo and active groups are shown in Table 6-1. There were two minor differences between the groups: significantly more participants were unemployed in the placebo (n=5/15) than the active (n=0/15) group (p = .04), and a significantly higher number of participants in the placebo group had GCSEs or vocational qualifications than the active (6 vs 1, p = .04). Aside from these minor differences the groups were similar on age, sex, IQ, medication status, other measures of education and employment, comorbid mental health conditions, and previous cannabis use. Baseline comparisons for the primary and secondary outcomes are shown in Table 6-2; there were no significant differences between the placebo and active groups on any of the outcomes.

Table 6-1: Baseline demographics

	N A/P	**Active[1] M(SD)/n**	**Placebo[1] M(SD)/n**	**t(*p*)**	**MW *z(p)***	***X*² (*p*)**	***Fishers Exact (p)***
Age (y/m)	15/15	36.91 (11.70)	38.90 (11.54)	0.47 (0.64)	-	-	-
Sex (f/m)	15/15	6/9	5/10	-	-	0.14(0.71)	-
IQ	15/15	111.87 (12.59)	114.93 (14.79)	0.61 (0.55)	-	-	-
Medication (med/unmed)	15/15	8/7	7/8	-	-	0.13 (0.72)	-
Income (£)	12/12	32,257.75 (26,461.55)	32,635.67 (42,998.28)	-	-1.24 (0.21)	-	-
Employment status							
Employed	15/15	14	9	-	-	-	.08
Unemployed	15/15	0	5	-	-	-	.04*
Full-time student	15/15	1	1	-	-	-	1.00
Highest level of education							
No qualifications	15/14	1	0	-	-	-	1.00
GCSE/Vocational	15/14	1	6	-	-	-	**.04***
AS-Level/A-Level	15/14	3	1	-	-	-	.60
Degree (undergrad/ postgrad)	15/14	10	7	-	-	0.83(0.36)	-
One or more comorbid condition (MINI)	15/15	10	12	-	-	-	0.68
Cannabis use							
No use	15/15	5	5	-	-	-	1.00
Daily (present)	15/15	3	1	-	-	-	0.60
Daily (past)	15/15	1	3	-	-	-	0.60
Weekly (past)	15/15	1	0	-	-	-	1.00
Monthly (present)	15/15	0	1	-	-	-	1.00
Monthly (past)	15/15	3	3	-	-	-	1.00
Yearly (past)	15/15	2	2	-	-	-	1.00

Note. N A/P = Number of participants in the Active/Placebo group, y/m = years/months, f/m = female/male; *p < .05

Table 6-2: Baseline comparisons of primary and secondary outcomes

	N A/P	Active M(SD)	Placebo M(SD)	*t*(*p*)	MW *z*(*p*)
Primary outcome					
Qb Test	14/14	1.73 (0.66)	1.71 (0.95)	-0.06 (0.95)	-
Post-hoc					
Qb Activity	14/14	2.66 (0.79)	2.61 (0.87)	-0.18 (0.86)	-
Qb Inattention	14/14	1.58 (1.31)	1.71 (1.46)	0.25 (0.81)	-
Qb Impulsivity	14/14	0.95 (1.32)	0.82(1.25)	-0.26 (0.79)	-
Secondary outcomes					
ADHD Symptoms					
CW Inattention	15/15	27.27(4.42)	27.33(6.17)	0.03(0.97)	-
CW Hyp/Imp	15/15	19.4(4.24)	19(7.44)	-0.18(0.86)	-
CW EL	15/15	15.6(5.53)	19.07(6.26)	1.61 (0.12)	-
Cognition					
SART CE	15/14	36.53 (16.24)	32.71 (16.55)	-0.63 (0.54)	-
SART OE	15/14	51.8 (53.67)	41 (53.10)	-	-1.31 (0.19)
SART RTV	15/14	186.85 (51.56)	156.32 (59.88)	-1.47 (0.15)	-
SART CV	15/14	0.55 (0.20)	0.43 (0.16)	-1.73 (0.09)	-
Emotional lability					
CNS-LS	15/15	30.67 (15.43)	30.2 (16.95)	-0.08 (0.94)	-
ALS	15/15	22.33 (11.14)	22.2 (9.51)	-0.04 (0.97)	-
Functional impairment					
WFIRS Total	15/15	1.17 (0.52)	1.11 (0.33)	-0.36 (0.72)	-

Note. N A/P = Number of participants in the Active/Placebo group, OE = Omission errors, CE = Commision errors, RTV=Reaction time variability, CV= Coefficient of variation (RTV/MRT)

6.5.4 Numbers analysed

For the primary outcome (the QB Test): the ITT analysis included 15 participants in the active and 11 in the placebo group. The per-protocol analysis included 14 participants in the active and 11 in the placebo group.

6.5.5 Drop-outs

Analysis of drop-outs for the per-protocol analysis shows that although there were more drop-outs in the placebo (n=4/15) than active (n=1/15) group this did not differ significantly (p=0.33).

6.5.6 Dosing

Average doses of the placebo and active medication in the final 4 weeks of the study were compared. Participants in the active group took significantly less sprays than those in the placebo group (mean = 4.73 vs 8.48 respectively, p = .02) (see Table 6-3).

Table 6-3: Average dose per day taken by participant in the final 4 weeks of the study (obtained from the study diary).

	Active (n=11) M(range, SD)	**Placebo (n=11) M(range, SD)**	***t*(*p*)**
Dose p/d	4.73 (1-13, 3.33)	8.48 (2.1-14, 3.80)	2.46 **(0.02)****

M=Mean, *SD* = Standard Deviation

** $p < .05$

6.5.7 Outcomes and estimation

6.5.7.1 ITT analysis

The intent-to-treat analysis is shown in Table 6-4. In the active group, the primary outcome performance on the QB Test showed an indication of improvement (p = 0.16, 95% CI = -0.40 to 0.07, d=0.59). Post-hoc comparisons of the individual domains of activity, inattention, and impulsivity showed this was driven mainly by reductions in activity during the Qb Test, though this effect was non-significant (p=0.24, d = 0.50).

For the secondary outcomes, a nominally significant reduction in symptoms of hyperactivity/impulsivity (p = .03, d=0.90) was found, although this did not withstand adjustment

for multiple testing (p> .004). Indications of improvement in symptoms of inattention (p = 1.0, d=0.66) and emotional lability (CNS-LS: p = 0.11, d = 0.65; ALS: p = 0.19, d = 0.52) were found in the active group although both failed to reach significance. Improvement on EL measured by the CAARS/WRAADS showed no indication of improvement. In the SART task there was an indication of improvement in the active group for CV (p = 0.14, d = 0.64). Treatment effects were not found for any of the other SART performance measures. There was no evidence of improvement in functional impairment.

6.5.7.2 Per-protocol analysis

The secondary per-protocol analysis is shown in

Table **6-5**. In the active group, the primary outcome (performance on the Qb Test) showed an improvement at trend level (p=0.06, d = 0.84). Post-hoc analysis showed this effect appeared to be driven mainly by an indication of a reduction in activity during the Qb Test (p = 0.15, d = 0.63) and potentially improvements in impulsivity (p = 0.23, d = 0.52) although both these effects were non-significant.

For the secondary analysis, a nominally significant improvement in the active group in symptoms of hyperactivity/impulsivity (p=0.02, d =1.03) was found, although this did not withstand adjustment for multiple testing (p> .004). An improvement at trend level was also found for symptoms of inattention (p=0.09, d =0.74). For emotional lability, there was an indication of improvement on the CNS-LS (p=0.13, d =0.67), but not on the ALS or on EL measured by the CAARS/WRAADS. In the SART task there was an indication of improvement in the active group for CV (p = 0.14, d = 0.67), but no other treatment effects on the SART performance measures were shown. There was no indication of improvement in functional impairment.

6.5.7.3 Sensitivity analysis

Monotone imputation (MAR assumption)

Outcomes with MI conducted using the monotone imputation are reported in Appendix E, Table AE-2. There was no indication of an effect for the primary outcome (the Qb Test) (p = 0.69). Post-hoc analysis showed no effects for Qb activity, inattention or impulsivity (all p > .05).

For the secondary outcomes, a nominally significant reduction in symptoms of hyperactivity/impulsivity remained (p=0.02), although this did not withstand adjustment for multiple testing (p< .004). A trend for improvement was found for inattention (p=0.08) and an indication of improvement for emotional lability (measured with the CNS-LS (p=0.12)). Treatment effects were not present for: emotional lability (measured with the CAARS/WRAADS and ALS), any measures from the SART (commission, omission errors, RTV, CV), or functional impairment.

Arbitrary (FCS) imputation (MAR assumption)

Outcomes with MI conducted using the arbitrary (FCS) imputation are reported in Appendix E, Table AE-3. As before, there was no indication for an effect on the primary outcome (p > .05). For the secondary outcomes, the treatment effect on hyperactivity/impulsivity was at trend (p=0.08), and treatment effects on emotional lability (measured with the CNS-LS) were now at trend (p=0.09). All other trends and non-significant effects remained the same as when using the monotone imputation method.

Imputation under the MNAR assumption

Outcomes under the MNAR assumption remained the same as those under the MAR assumption (monotone imputation method). This indicates the MAR assumption could be accepted (See Appendix E, Table AE-4).

Table 6-4: Intent to treat analysis

	Pre-treatment (M(SD))			Post-treatment (M(SD))			Time x treatment				
	Active	**Placebo**	**N A/P**	**Active**	**Placebo**	**N A/P**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
Primary outcome											
Qb Test	1.73 (0.66)	1.71 (0.95)	14/ 11	1.32 (0.53)	1.46 (0.91)	15 / 11	-0.17	0.12	-0.40 to 0.07	0.16	0.59
Post-hoc											
Qb Activity	2.66 (0.79)	2.61 (0.87)	14/14	2.13 (1.09)	2.43 (0.87)	15/11	-0.22	0.19	-0.61 to 0.16	0.24	0.5
Qb Inattention	1.58 (1.31)	1.71 (1.46)	14/14	0.91 (0.90)	1.05 (1.10)	15/11	-0.11	0.17	-0.46 to 0.25	0.55	0.25
Qb Impulsivity	0.95 (1.32)	0.82 (1.25)	14/14	0.91 (0.87)	0.91 (1.37)	15/11	-0.13	0.22	-0.57 to 0.31	0.55	0.25
Secondary outcomes											
ADHD Symptoms											
CW Inattention	27.27 (4.42)	27.33 (6.17)	15/ 15	17.60 (8.87)	21.92 (7.52)	15/13	-2.41	1.43	-5.34 to 0.52	0.10	0.66
CW Hyp/Imp	19.40 (4.24	19.00 (7.44)	15/ 15	10.20 (5.58)	13.85 (7.46)	15/ 13	-2.45	1.07	-4.65 to -0.26	**0.03****	0.90
CW EL	15.60 (5.53)	19.07 (6.26)	15/ 15	8.47 (5.45)	12.08 (5.75)	15/ 13	-0.16	1.17	-2.56 to 2.24	0.89	0.05
Cognition											
SART CE	36.53 (16.24)	32.71 (16.55)	15/ 14	28.93 (17.41)	23.00 (15.55)	15/ 10	-1.23	2.19	-5.73 to 3.27	0.58	0.24
SART OE	51.80 (53.67)	41.00 (53.10)	15/ 14	43.07 (51.95)	20.20 (28.56)	15/ 10	2.11	5.99	-10.18 to 14.40	0.73	0.15
SART RTV	186.85 (51.56)	156.32 (59.88)	15/ 14	177.04 (58.41)	134.60 (48.64)	15/ 10	-1.09	7.88	-17.25 to 15.06	0.89	0.06
SART CV	0.55 (0.20)	0.43 (0.16)	15/ 14	0.49 (0.20)	0.38 (0.16)	15/ 10	-0.03	0.02	-0.07 to 0.01	0.14	0.64
Emotional lability											
CNS-LS	30.67 (15.43)	30.20 (16.95)	15/ 15	20.13 (15.46)	27.92 (12.44)	15/ 13	-3.77	2.28	-8.44 to 0.89	0.11	0.65
ALS	22.33 (11.14)	22.20 (9.51)	15/ 15	15.40 (9.49)	21.38 (9.14)	15/13	-2.92	2.19	-7.41 to 1.58	0.19	0.52
Functional impairment											
WFIRS Total	1.17 (0.52)	1.11 (0.33)	15/15	0.83 (0.49)	0.77 (0.26)	15/11	-0.02	0.09	-0.20 to 0.15	0.81	0.10

** $p \leq .05$ (nominally significant). Note. A lower score indicates an improved outcome for all measures, M = Mean, SD = Standard deviation, SE = Standard error, Est = Estimate, OE = Omission errors, CE = Commision errors, RTV=Reaction time variability, CV= Coefficient of variation (RTV/MRT)

Table 6-5: Per-protocol analysis

	Pre treatment (M(SD))			**Post-treatment (M(SD))**			**Time x treatment**				
	Active (M(SD))	**Placebo (M(SD))**	***N* (A/P)**	**Active (M(SD))**	**Placebo (M(SD))**	***N* (A/P)**	**Est**	**SE**	**Est 95% CI**	***p***	***d***
Primary outcome											
QB Test	1.75 (0.68)	1.41 (0.93)	13/10	1.30 (0.55)	1.46 (0.91)	14/11	-0.24	0.12	-0.48 to 0.01	**0.06***	0.84
Post-hoc											
Qb Activity	2.68 (0.83)	2.41 (0.93)	13/10	2.09 (1.12)	2.43 (0.87)	14/11	-0.29	0.20	-0.70 to 0.11	0.15	0.63
Qb Inattention	1.47 (1.30)	1.32 (1.43)	13/10	0.86 (0.91)	1.05 (1.10)	14/11	-0.16	0.18	-0.54 to 0.21	0.38	0.38
Qb Impulsivity	1.10 (1.25)	0.50 (1.13)	13/10	0.95 (0.89)	0.91 (1.37)	14/11	-0.27	0.22	-0.72 to 0.18	0.23	0.52
Secondary outcomes											
ADHD Symptoms											
CW Inattention	27.21 (4.58)	25.91 (6.52)	14/11	16.86 (8.71)	21.00 (7.86)	14/11	-2.72	1.55	-5.93 to 0.48	**0.09***	0.74
CW Hyp/Imp	19.50 (4.38)	16.18 (6.42)	14/11	9.93 (5.69)	12.27 (7.00)	14/11	-2.83	1.16	-5.23 to -0.44	**0.02****	1.03
CW EL	15.07 (5.33)	17.64 (6.34)	14/11	7.50 (4.11)	11.00 (5.53)	14/11	-0.47	1.26	-3.07to 2.13	0.71	0.16
Cognition											
SART CE	36.07 (16.75)	28.55 (15.69)	14/11	28.57 (18.01)	23.00 (15.55)[a]	14/10	-1.57	2.28	-6.28 to 3.15	0.50	0.30
SART OE	45.43 (49.46)	35.27 (44.54)	14/11	38.79 (51.09)	20.20 (28.56)[a]	14/10	2.33	6.08	-10.24 to 14.91	0.70	0.17
SART RTV	184.48 (52.65)	146.18 (49.91)	14/11	176.35 (60.55)	134.60 (48.64)[a]	14/10	-1.25	8.08	-17.97 to 15.47	0.88	0.07
SART CV	0.55 (0.21)	0.38 (0.13)	14/11	0.50 (0.21)	0.38 (0.16)[a]	14/10	-0.03	0.02	-0.08 to 0.01	0.14	0.67
Emotional lability											
CNS-LS	30.29 (15.94)	29.73 (16.87)	14/11	18.50 (14.64)	25.09 (11.27)	14/11	-3.57	2.25	-8.24 to 1.09	0.13	0.67
ALS	22.50 (11.54)	23.91 (10.11)	14/11	14.79 (9.54)	20.36 (8.00)	14/11	-2.08	2.41	-7.07 to 2.90	0.40	0.36
Functional impairment											
WFIRS Total	1.15 (0.54)	1.01 (0.26)	14/11	0.78 (0.47)	0.77 (0.26)	14/11	-0.06	0.09	-0.25 to 0.12	0.48	0.30

Note. A lower score indicates an improve outcome for all measures, M = Mean, SD = Standard deviation, SE = Standard error, Est = Estimate, * Trend, ** $p < .05$ (nominally significant)

6.5.8 Assessment of blinding

Fourteen participants in the active group and thirteen in the placebo group were asked to guess the medication they were taking. In the active group 13 (93%) and in the placebo group 11 (85%) correctly guessed their allocation status. There was no difference in the correct guess rate between the two groups (Fisher's exact $p = 0.60$).

6.5.9 Qualitative feedback

Qualitative feedback from the participants in the active medication group (in response to the open question "How has the medication made you feel overall?") is detailed in Table 6-6 and Appendix E, Table AE-5. There were more reports of positive effects (n=36) than negative (n=17). Positive effects generally included: feeling calmer (n=8), improved focus/concentration (n=6), more relaxed (n=4), improved sleep (n=4), and reduced anxiety (n=2). For example, participants stated:

"Sustained periods of concentration which would have been difficult to achieve unmedicated. Effects were positive overall. Didn't like the taste, but no dry mouth or insomnia like I get from usual ADHD meds. Slept well and woke rested - major benefit. Stayed on tasks for longer, generally would prefer to take over ADHD meds."

"Just feels like it had an effect on me. Everything becomes more focused, clearer, thoughts are clearer. Also lessens anxiety I have from time to time. Less of a come down than Ritalin and effects probably last for 1 to 2 hours."

Negative feedback was generally regarding the sedative effects of the medication (n=6) and atypical feelings (e.g. odd thoughts, detachment) (n=5) (which could have been at higher doses). For example one participant stated the side-effects to include:

"Odd thoughts on 8+ sprays a day, feeling detached, couldn't be bothered, in my own world and sometimes forgetful."

Table 6-6: Qualitative feedback from participants in the active group (in response to the open question "How has the medication made you feel overall?")

Positive	Frequency reported	Negative	Frequency reported
Calm	8	Spacey	1
Clearer	2	Slows you/thoughts down	3
Focused/improved concentration	6	Slightly stoned	1
Relaxed	4	Does not help attention	1
Energetic	1	No effect	1
Improved sleep	4	Mouth ulcers	1
Improved side-effect profile to ADHD medication (no insomnia/dry mouth/less of a 'come down')	2	Sedating	3
Felt better/happier	1	Felt a bit odd	1
Reduced anxiety/panic	2	Vulnerability to anxiety	1
Woke-up rested	2	Altered clarity/focus of vision	1
More engaged in activities and work	1	Headaches	1
Better able to sustain interest	1	At a higher dose: Odd thoughts, detached, 'couldn't be bothered, forgetful, in their own world, talkative confused, restless, 'trapped in own head', 'saying wrong things'	2
Steadied thought processes	1	-	
Slightly improved productivity	1	-	
Total	36		17

6.5.10 Adverse events

Two serious adverse events occurred during the study, one of which was thought to be due to study medication (both were reported to GW Pharma, King's College Research and Development Office, and NRES Committee London-London Bridge). One participant on the active medication (two weeks into the trial) reported sudden onset of muscular seizures/spasms and stopped taking the medication. This has not been previously reported with Sativex and therefore may have been an atypical reaction to the medication or may have reflected an anxiety attack. The second participant was taking the placebo medication (15 days into the trial) and experienced an increased heart rate, tightness of chest and breathing. This required hospital investigation with no obvious cause

identified. As the participant was taking the placebo it was very unlikely that this was due to the medication. A mild adverse event was experienced by three participants in the active group. Two rang the emergency phone to report that they were experiencing light-headedness, reporting this as feeling “very weird” and “spaced out”. One participant had taken two sprays at once in the first week of the trial. The other participant was in the fourth week of the trial and had stopped the medication for 6 days as he had a minor operation. He experienced symptoms after taking two sprays with a gap of a few hours in between. The symptoms in both participants resolved after a few hours and they continued with the trial. One participant reported diarrhoea and stopped taking the medication for 4 days, on resuming the medication there were no complications and this may have been unrelated.

Table 6-7 shows a comparison of the side effects reported by the active and placebo group on day 28 (although for 4 participants this was reported on day 14 and for 1, day 12 due to their either dropping out or difficulty in contacting). There were no significant differences in side effects between the two groups ($p > .05$), although there were trends for those in the active group to report more dizziness (z=-1.80, p=0.07) and sadness (z=-1.85, p=0.07).

Table 6-7: Comparison of side effects of the active versus placebo medication (rated using the Adverse Events Scale)

	N (A/P)	Active (M(SD))	Placebo (M(SD))	*Z*(*p*)
Headache	15/15	0.07 (0.26)	0.2 (0.41)	1.06 (0.29)
Dryness of the skin	15/15	0.27 (0.46)	0.4 (0.91)	-0.20 (0.84)
Dryness of the eyes	15/15	0.13 (0.35)	0.13 (0.52)	-0.52 (0.60)
Dryness of the mouth	15/15	0.4 (0.63)	0.33 (0.62)	-0.36 (0.72)
Thirst	15/15	0.6 (0.91)	0.27(0.70)	-1.44 (0.15)
Sore throat	15/15	0.33 (0.62)	0.07 (0.26)	-1.47 (0.14)
Dizziness	15/15	0.2 (0.41)	0.00 (0.00)	-1.80 (0.07)
Nausea	15/15	0.00 (0.00)	0.00 (0.00)	-
Stomach aches	15/15	0.00 (0.00)	0.07 (0.26)	1.00 (0.32)
Vomiting	15/15	0.00 (0.00)	0.07 (0.26)	1.00 (0.32)
Sweating	15/15	0.00 (0.00)	0.13 (0.52)	1.00 (0.32)
Appetite reduction	15/15	0.00 (0.00)	0.2 (0.77)	1.00 (0.32)
Weight loss	15/15	0.07 (0.26)	0.13 (0.52)	0.05 (0.96)
Weight gain	14/15	0.29 (0.47)	0.13 (0.52)	-1.40 (0.16)
Diarrhea	15/15	0.00 (0.00)	0.00 (0.00)	-
Frequent urination	15/15	0.13 (0.35)	0.13 (0.35)	0.00 (1.00)
Tics	15/15	0.00 (0.00)	0.00 (0.00)	-
Sleep difficulties	15/15	0.2 (0.41)	0.4 (0.83)	0.51 (0.61)
Mood instability	15/15	0.2 (0.41)	0.2 (0.41)	0.00 (1.00)
Irritability	15/15	0.2 (0.41)	0.2 (0.41)	0.00 (1.00)
Agitation/excitability	15/15	0.33 (0.62)	0.2 (0.56)	-0.83 (0.40)
Sadness	15/15	0.47 (0.74)	0.07 (0.26)	-1.85 (0.07)
Heart palpitations	15/15	0.00 (0.00)	0.2 (0.77)	1.00 (0.32)
Sexual dysfunction	15/15	0.00 (0.00)	0.07 (0.26)	1.00 (0.32)
Feeling worse or different when the medication wears off (rebound)	15/10	0.13 (0.52)	0.2 (0.63)	0.30 (0.77)
Paranoia	6/8	0.00 (0.00)	0.00 (0.00)	-

Note. A higher score represents increased side-effects.

6.6 Discussion

To our knowledge, this is the first randomised controlled trial of a cannabinoid medication (Sativex) in ADHD. Our objective was to provide preliminary data on the relationship of short-term treatment with a cannabinoid-based medication on cognitive and behavioural measures in adults with ADHD. Although there was no significant effect for the primary outcome, performance on the Qb Test, trend effects were seen for the ITT and per-protocol analyses; with medium to large effect sizes of d= 0.59 and d= 0.84 respectively. In both the ITT and per-protocol analysis, these effects appeared to be driven by reductions in the activity score (d = 0.5 and d = 0.63 respectively). In the per-protocol analysis effects also appeared to be driven by reductions in commission errors (d=0.52). However, effects on the Qb test did not withstand the sensitivity analyses (under both the MAR and MNAR assumption), whereby all missing data was imputed. Given that data was missing mainly from the placebo group, this suggests that potential bias towards positive effects should be taken into account when interpreting results.

For the secondary exploratory analyses for ADHD symptoms, a nominally significant treatment effect was found for symptoms of hyperactivity/impulsivity in both the ITT and per-protocol analyses with large estimates of effect sizes of d = 0.90 and d =1.03 respectively. The effect on hyperactivity/impulsivity, although weaker, remained in the sensitivity analysis. Symptoms of inattention also showed a non-significant trend to improvement in the ITT and per-protocol analyses, with medium effect sizes at d=0.66 and d=0.74 respectively. These trends remained in the sensitivity analysis. For emotional lability, there was a non-significant trend for improvement in the ITT analysis (measured with the CNS-LS (d = 0.65) and ALS (d = 0.52)), which was weaker in the per-protocol analysis (observed only for the CNS-LS). In the sensitivity analysis, trends remained for the CNS-LS only. For cognition measured with performance on the SART task, there was a non-significant trend for improvements in the coefficient of variation (CV) for both the ITT and per-protocol analysis at medium effect (d = 0.64 and d = 0.67 respectively). This trend did not remain in the sensitivity analysis. No other effects were observed for the SART. No treatment effects were observed for functional impairment in either the ITT, per-protocol or sensitivity analyses. None of the significant effects for either the primary or secondary outcomes withstood correction for multiple testing.

As a whole this study failed to identify significant changes from baseline to endpoint for the primary and secondary variables, using conventional levels of significance and adjusting for the number of cognitive and behavioural variables investigated. Nevertheless the findings are of interest because effects were in the expected direction for a positive effect for most variables, some showed nominal significance, and estimates of effect size were moderate to large, raising the expectation that further larger investigations would show significant effects. On this basis, the findings provide preliminary evidence that Sativex may reduce hyperactivity/impulsivity and perhaps inattention in adults with ADHD. Evidence for improvements in cognition measured by the Qb Test and the SART and emotional lability were weaker although still appeared to be promising. There was no evidence of change in functional impairment, although the short duration of the study may have not been appropriate for measuring any change in functional impairment.

Greater estimates of effect size were found in the per-protocol than the ITT analysis. The per-protocol analysis includes participants who adhered strictly to the protocol. The ITT analysis is considered to be less prone to bias by including all participants who were randomised (and for whom follow-up data were available) (Gupta, 2011). Therefore effect sizes found in the per-protocol analysis are generally larger than those found in the ITT. Effects found in *both* the per-protocol and ITT analysis provide preliminary evidence for the presence of a treatment effect. Therefore this study found evidence for potential effects of Sativex to improve symptoms of hyperactivity/impulsivity. Indications of improvement for inattention and the Qb Test were stronger in the per-protocol than the ITT analysis, providing overall weaker evidence. However, given the small sample size these initial findings are promising.

The greatest improvement in this study was found for symptoms of hyperactivity/impulsivity, with treatment effects on inattention at trend. One possible explanation for improvement in hyperactivity/impulsivity may be due to the calming and anxiolytic effects of cannabidiol (CBD) and Δ9-THC, the two cannabinoids found in Sativex (Zuardi et al., 2006). Research administering acute doses of these cannabinoids (in placebo-controlled designs) in typically developing controls and cannabis users find effects to include feeling “calm” and “relaxed” (D’Souza et al., 2008; Zuardi et al., 2006). The calming effect of cannabis in those who are non-ADHD symptomatic may be impairing and sedating, whereas those with ADHD, who have increased levels of restlessness and impulsivity,

may show differences in response. The calming effect may help to alleviate these symptoms and allow for improved outcomes. Reduction in symptoms of hyperactivity/impulsivity may also lead to 'top-down' improvements in inattention due to better ability to remain still and therefore focus (which could account for the larger effect size found for hyperactivity/impulsivity). Differences in response may be due to the proposed effect of cannabinoids on dopamine levels and differences in these levels between adults with ADHD and controls (this will be discussed later). The theory of differential response to Sativex between adults with ADHD and controls highlights the need to include a control group in either a future treatment trial, or in a study that administers acute doses of Sativex, in order to clarify this proposal.

This study has indicated that Sativex could have a positive effect on cognition. Preliminary evidence for effects on the Qb test were found for improvements in activity and potentially commission errors, and for the SART for improvements in CV, although these trends for improvement did not withstand the sensitivity analysis whereby data was imputed for all participants. This suggests that the increased rate of drop-outs in the placebo than the active group may have biased results in favour of the treatment group. Despite this limitation, multiple imputation is conservative therefore these results may be indicative of a potential treatment effect on these measures. A larger trial where a greater number of participants are randomised to the placebo arm (to account for potential drop-out) will be required to provide more definitive evidence.

Indications of improvements in commission errors (inhibition) and RTV (indexed by CV) is a somewhat surprising result given that reviews have associated cannabis use with impaired cognitive function including inhibitory deficits (Crean, Crane, & Mason, 2011; Solowij & Battisti, 2008). Acute Δ9-THC administration has been associated with significant impairments in response inhibition (McDonald et al., 2003; Ramaekers et al., 2009), commission and omission errors and reaction time (McDonald et al., 2003; Ramaekers et al., 2009; Ramaekers et al., 2006). Although this is not a robust finding as for example, the same study that found deficits in response inhibition did not find deficits in omission and commission errors (McDonald et al., 2003). Despite this mixed evidence, to our knowledge there is no evidence, from a sample of both naive and regular users, that cannabinoids could potentially *improve* cognitive function, as suggested by the findings in this study.

This preliminary evidence to suggest that cannabinoids improve behavioural and potentially cognitive function in adults with ADHD could be explained in terms of individual differences in response to cannabinoids. The ADHD population may represent a subgroup of people who more commonly respond positively to cannabinoid medication than would a sample from the general population. The presence of individual differences in response to cannabis is well established (Block, Erwin, Farinpour, & Braverman, 1998; Green, Kavanagh, & Young, 2003). For example, experimental studies of intravenous Δ9-THC administration in typically developing participants find some but not all (~40-50%) will experience psychotic symptoms (Englund et al., 2012; Morrison et al., 2009).

Subjective accounts from patients in this trial, who commonly reported improvements in focus and concentration, gives support to the proposal that adults with ADHD respond more positively to cannabinoids than those in the general population. The high levels of ADHD symptoms seen in the ADHD population may moderate the effects of cannabinoids on behavioural and potentially cognitive function, leading to a more positive response (Meier et al., 2012; Mokrysz et al., 2014). Interestingly, heavy cannabis users have been found to show less cognitive impairment following cannabis use than occasional users (D'Souza et al., 2008; Ramaekers et al., 2009), although one possibility is that heavy users have developed tolerance to the drug. This explanation is unlikely for the current trial which may have been too short for the development of tolerance. A recent trial found no evidence of tolerance development after 1-3 years of Sativex use in patients with multiple-sclerosis (Serpell, Notcutt, & Collin, 2013). Furthermore a baseline level of tolerance to cannabinoids is unlikely, given that the majority of participants were either cannabis naive (33%), or were not currently using the drug (50%), with only 13% currently using on a daily basis. Therefore regular users who may have a tolerance to the drug were in a minority. An alternative explanation for the potential beneficial effect of cannabinoids on behaviour and cognition is that those who are drawn to cannabis may be 'innately' protected from the negative effects of the drug (D'Souza et al., 2008). Given the high incidence of ADHD which has been found in those who abuse or are dependent on cannabis (Dennis et al., 2004), the theory of being 'innately' protected from the negative effects of cannabis could be applicable to those with ADHD.

Potential improvements in cognition could also be linked to the high concentration of the cannabinoid cannabidiol (CBD) found in Sativex (which contains Δ9-THC and CBD in a 1:1 ratio). Studies in humans have suggested Δ9-THC to be responsible for the cognitive deficits induced by cannabis and that CBD may protect against these impairments (Englund et al., 2012; Morgan et al., 2012; Morgan et al., 2010). A potential explanation is that possible cognitive improvements are a combination of the CBD concentration and more positive response to the cannabinoid medication in adults with ADHD. Investigation of the differential effects of CBD and Δ9-THC in adults with ADHD would be important to clarify this proposal.

The physiological effects of cannabis may also underpin the theory that those with ADHD show individual variation in response to cannabinoids. One mechanism of action of cannabis is thought to be through alterations in dopamine. Abnormalities in dopamine levels, particularly in the striatum, is a common theory of ADHD (del Campo et al., 2012; Hesse et al., 2009; Krause et al., 2005; Krause et al., 2000), evidenced mainly by the positive effects of dopamine-enhancing stimulant medication (Leonard et al., 2004; Volkow et al., 2002). Striatal dopamine is thought to modulate the endocannabinoid system (ECS) (Centonze et al., 2009), and a number of studies have found Δ9-THC administration to increase dopamine in the striatum (Bossong et al., 2009, 2015; Kuepper et al., 2013) and in other brain areas implicated in ADHD, such as the prefrontal cortex in animal studies (Chen et al., 1990; Pistis et al., 2002), and the right middle frontal gyrus and left superior frontal gyrus in human studies (Stokes et al., 2010). Other studies have, however, contradicted the dopamine-agonist effects of cannabis (Barkus et al., 2011; Bloomfield et al., 2014; Stokes et al., 2009). Further research into the mechanism of action of Sativex is therefore important in order to clarify the effects on dopamine.

Although this study found only nominally significant effects or trends, effect sizes on ADHD symptoms were medium to large (d=0.7-1), which is equivalent to those found for stimulant medication on ADHD symptoms (d ~ 0.6-1.0) (Faraone & Buitelaar, 2010; Faraone & Glatt, 2010; Faraone et al., 2004; Mészáros et al., 2009). Effects on cognition (Qb impulsivity and SART CV) (d=0.5-0.7) were also equivalent to that found for stimulant medication on cognition (d =~0.2-0.6) (Coghill et al., 2014). These are promising results, since the lack of significant effects may be due to the small sample size employed in this study. Power calculations, taking the smallest effect on

ADHD symptoms (d = 0.7), indicate a total sample size of 68 participants would be required (α = 0.05, power = 0.81) to detect an effect at a nominal level of significance, and 118 participants after correction for multiple testing (α = 0.004, power = 0.80). For cognition, a total sample of 128 participants would be required (d = 0.05, α = 0.05, power = 0.80), and 226 after correction for multiple testing. A future trial should include fewer outcome measures in order to increase the α after correction for multiple testing and aim to recruit 150-200 participants in order to gain a definitive answer to whether Sativex may improve symptoms and cognition in ADHD.

Results here have provided objective preliminary evidence which goes towards explaining the subjective reports of adults with ADHD. Such patients have reported an improvement in their symptoms following cannabis use including feeling reduced restlessness and distractibility with improved concentration and ability to sustain focus. Subjective accounts from patients in the active group are reported in Table 6-6 and Appendix E, Table AE-5. Positive feedback was more than double the negative feedback and most commonly included: feeling calmer, improved focus/concentration, more relaxed, improved sleep and reduced anxiety. Negative feedback was generally regarding the sedative effects of the medication and atypical feelings (e.g. odd thoughts, detachment) at higher doses. Furthermore, we did not find any change in impairment between the active and placebo groups. This is important given that cannabis is often associated with functional impairment in the general population. The results here are therefore in line with the theory that individuals with ADHD may be using cannabis to self-medicate (Horner & Scheibe, 1997; Wilens, 2004).

Here we have given preliminary evidence that Sativex could be an alternative treatment for adults with ADHD. Given the high rates of cannabis use in ADHD, it is important that a licensed, safer medication is investigated. The main psychoactive chemicals in cannabis are Δ9-THC and CBD. As previously discussed, the more harmful effects of cannabis use such as the association between cannabis and psychosis and cannabis and cognitive impairments, has been linked to Δ9-THC (D'Souza et al., 2009; Englund et al., 2012). However, CBD is thought to have more protective effects and has been found to reduce Δ9-THC induced psychotic symptoms and cognitive impairments (Englund et al., 2012). Recent research has found the link between cannabis and psychosis may be specific to cannabis that is high in Δ9-THC (known as sinsimella (skunk)), but not

that which contains similar levels of Δ9-THC to CBD (known as cannabis resin ('hash')) (Di Forti et al., 2015). Sativex contains Δ9-THC and CBD in a 1:1 ratio, whereas the UK black market is currently dominated by Sinsemilla, with levels of Δ9-THC appearing to rise year on year, and cannabis resin increasingly difficult to obtain (Potter et al., 2008). The prescription of Sativex would therefore provide a safer alternative to the black market cannabis that is most likely being used in the ADHD population.

A number of important limitations must be considered when taking the current results into account. One of the main limitations of this study is that, although participants and study staff were blinded throughout the trial, correct rates of guessing allocation status were high with 93% of participants in the active and 85% in the placebo group guessing correctly (albeit responses from 3 participants were missing). This is a problem inherent to the use of a cannabinoid medication as firstly, the majority of participants having previously used cannabis, were therefore familiar with its effects and secondly, the effects of the spray being noticeable even at a low dose. Lack of blinding is associated with exaggerated estimates of intervention effects (Pildal et al., 2007). However greater bias is observed in trials with more subjective outcomes (Wood et al., 2008). Therefore failure of the blind may have biased the behavioural outcomes (e.g. ADHD symptoms) more so than cognitive performance. Potential exaggeration of treatment effects must be taken into account when interpreting our results. Future trials are vital and should compare Sativex to current ADHD stimulant medication to reduce the chance of breaking the blind.

A further important limitation to this study is that of a greater number of drop-outs in the placebo than the active group. Despite sensitivity analysis indicating no difference between outcomes under the MNAR and MAR assumption (meaning that the MAR assumption can be accepted), increased drop-out in the placebo group is indicative of non-random drop-out due to lack of treatment effect, although this is not clear from the reasons given by the participants who dropped-out (see Section 6.5.10 'adverse events'). Therefore, although an increased rate of drop-out in the placebo group may be non-random, due to lack of benefit from the study medication, we cannot say for certain that this is the case. However the possibility of non-random drop-out in the placebo group cannot be ruled out. This is especially given that participants were required to come off their ADHD medication for the duration of the trial, which may have been more difficult for those who

were not deriving benefit from the study medication, although of the two participants who were lost to follow-up, only one was currently taking ADHD medication. Despite this, data imputation in the sensitivity analysis gave an overall negative effect for the Qb Test. This indicates that increased drop-out in the placebo compared to the active group may have inflated effect size for the Qb test in the ITT and per-protocol analysis due to increased power in the active group. Although it is important to note that an increased drop-out rate in the placebo group supports our finding of a treatment effect indicating that those in the active group were more likely to derive benefit from the study medication, and therefore to remain in the trial.

Another limitation to this trial is that the optimum dose of Sativex may not have been used by every participant. This experimental pilot study was, to our knowledge, the first study examining Sativex as a treatment for ADHD. Therefore at the start of the trial the required dose in this group of patients was unknown. In Section 6.4.5.2 we discuss how it quickly became clear that the titration schedule recommended by GW Pharma was too high (the schedule increased to 14 sprays per day whereas the average number of sprays taken in the active group was 4.7 (range 1-13)). Participants were verbally informed at the baseline session and also during regular telephone monitoring (on days 4, 8, 12, 14 and 28) that they should remain at a lower dose if they were finding an effect of the medication. However at the final testing session a small number of participants had taken a high dose for the majority of the trial, and verbally reported that they did not derive benefits. This may have been compounded by there often being difficulty in contacting participants in order to monitor their progress. Failure to maintain an optimum dose in every participant may have led to a reduced effect size in the active group. A new titration schedule which increases to a maximum dose of ~8-9 sprays per day but recommends an optimum dosage of 3-5 sprays should be developed for future trials.

Furthermore, participants in the placebo group on average took a significantly higher (around double) number of sprays a day than those in the active group. This could have introduced bias; overestimating effects in the placebo group due to a perceived 'dosing' effect. However due to the failure of the blind this is unlikely. As advised above, a future study should use a titration schedule which increases to 8-9 sprays instead of 14 sprays in the current study. This will reduce the

possibility of participants in the placebo group taking a dose that is considerably higher than that of the active group.

Two serious adverse events (AE) occurred during the study. One participant in the active group experienced muscular seizures/spasms which may have been an atypical reaction to the medication or anxiety related. The second participant in the placebo group experienced cardiac problems. A meta-analysis of the safety of Sativex in patients with MS found a small number of participants to report cardiac problems in the placebo group (n=2/303). With such a small number of cardiac events it is unlikely but not implausible that the AE was due to the placebo medication. Although the side-effect rating scale showed no significant difference in side-effects between the placebo and active groups, three participants in the active group experienced a mild adverse event (AE) during the study. One had diarrhoea, and two participants experienced 'lightheadedness' after taking two sprays of Sativex either in quick succession or in the space of a few hours having not taken the spray for 6 days previously. These mild AEs (e.g. gastrointestinal complaints, dizziness) have been experienced in other clinical studies of Sativex (Johnson, Lossignol, Burnell-Nugent, & Fallon, 2013; Novotna et al., 2011; Nurmikko et al., 2007; Wade, Collin, Stott, & Duncombe, 2010) and are listed as a common side-effect of the medication. Future trials in adults with ADHD need to take the AEs related to dosage (i.e. feeling 'weird', lightheadedness) into account when developing the titration schedule. It must be stressed to participants that they should not take two sprays at once. Participants should also be advised that if they stop taking the medication for a number of days they will need to titrate back onto the spray (i.e. begin at 1 spray per day). The occurrence of only one serious adverse event in the active group suggests that as a whole the medication was well tolerated.

Another point to consider in the proposal that Sativex could be an alternative treatment for adults with ADHD is the potential for abuse of this drug and development of tolerance. The potential for abuse of Sativex at varying dosages was examined in a treatment trial with recreational cannabis users (Schoedel et al., 2011). At a dose of 4 sprays there was no more potential for abuse than the placebo medication. Higher doses of Sativex (8-16 sprays) showed more potential for abuse than placebo. Sativex may not therefore be suitable for those with a history of abuse. It is important to point out, however, that stimulant medication which is currently prescribed for adult ADHD is a

controlled drug which also has potential for abuse. Tolerance has been discussed previously with a study investigating the long-term use of Sativex finding no evidence of tolerance-dependence in patients with MS who remained on the spray for between to 1-3 years. This is a promising result, although given the different patient population, this would need to be assessed in adults with ADHD.

In conclusion, this experimental pilot study has given preliminary evidence for the efficacy of Sativex in treating the symptoms, and to a lesser extent cognitive deficits, in adults with ADHD. The medication was relatively well tolerated without causing any significant impairment. This corroborates the subjective accounts of patients who may be currently self-medicating with cannabis. This evidence has provided a basis for the suggestion that Sativex could be suitable as a treatment for those who either cannot tolerate or find current ADHD medication to be ineffective. A large trial (150-200 participants) comparing Sativex with current stimulant medications is vital in order to gain a more conclusive picture.

Chapter 7: Overall conclusions and future directions

7.1 Summary

Treatments for ADHD in the form of stimulants and atomoxetine have moderate to large effects on both symptoms and impairments, and provide good control of the condition for the majority of cases. Yet there remains a significant group for whom treatment is sub-optimal, either due to partial or no clinical response, or to adverse effects. This has led to the continued search by clinicians, researchers and patient's to find effective alternative treatments. The aim of this thesis was to investigate the effect of two such alternative treatments: Omega-3 Polyunsaturated Fatty Acids (*n*-3 PUFAs) and a cannabinoid medication (Sativex Oromucosal Spray) on ADHD symptoms and associated impairments of cognitive performance and emotional lability (EL).

This thesis presents four novel studies. A systematic review and meta-analysis of the effects of *n*-3 PUFA supplementation on cognition showed no evidence for an effect in the general population or in children with ADHD or with a related disorder. Marginal evidence for effects was found in those who were *n*-3 PUFA deficient. A further meta-analytic study found suggestive evidence of a small effect of *n*-3 PUFA supplementation on reducing emotional lability (EL) and oppositional behaviour in children with ADHD or with a related neurodevelopmental disorder. The thesis then presents the first randomised controlled trial (RCT) of *n*-3 PUFA supplementation in adults with ADHD. Baseline case/control comparisons showed the ADHD group to have significant impairments in cognitive performance, as well as severe levels of ADHD symptoms and EL. However, no differences in *n*-3 PUFA blood levels were found that might have indicated a role for dietary deficiency. Supplementation gave marginal (non-significant) evidence for improvements in inattention and potentially EL, but no apparent effects on cognition. The negative results should be interpreted with caution given a high drop-out rate, yet the findings suggest that any possible effects are likely to be small and might therefore lack clinical significance. The final part of this thesis presented the first RCT of a cannabinoid medication, Sativex Oromucosal Spray, in adults with ADHD. Sativex was found to lead to nominally significant improvements in hyperactivity/impulsivity, with a trend for improvements in inattention and to a lesser extent cognitive performance. The small sample size used in this preliminary investigation, combined with the moderate to large effects observed, provide a promising initial set of findings.

As a whole this thesis does not support the proposed efficacy of *n*-3 PUFA supplementation on cognition in either children or adults with ADHD. The trial and also meta-analysis data identifies possible effects on EL and inattention, yet any potential effects are likely to be small and may therefore lack clinical relevance. The evidence from the Sativex trial is more promising.

7.2 Overview of findings

Table 7-1 restates the original hypotheses from each chapter with a brief summary of the relevant findings and an indication of whether each hypothesis was supported. Of the 14 proposed hypotheses, one was supported, partial evidence was provided for five, weak evidence for one, and no evidence for seven. The following section provides a brief overview of the findings from each chapter.

Table 7-1: Summary of findings in relation to original study hypotheses (n.b. Support for hypothesis from low-high: No, Weak Evidence, Partially, Yes)

Hypothesis	Supported?	Specific Results
Chapter 2: Omega-3 polyunsaturated fatty acid supplementation and cognition: a systematic review and meta-analysis		
Primary hypotheses		
1) Omega-3 PUFA supplementation will improve cognitive performance in children with ADHD or with a related neurodevelopmental disorder (e.g. dyslexia) (ADHD + RND group).	No	• Supplementation did not lead to improvements in any of the cognitive performance measures in children with ADHD+RND.
2) Omega-3 PUFA supplementation will improve cognitive performance in typically developing children and adults (TD group).	No	• Supplementation did not lead to improvements in any of the cognitive performance measures in the TD Group.
Secondary hypotheses (subgroup analysis)		
3) Omega-3 PUFA supplementation will improve cognitive performance in the ADHD+RND/TD group in those who are *n*-3 PUFA deficient.	Partially	• Supplementation in those who were *n*-3 PUFA deficient led to improvements in short-term memory across the TD and ADHD+RND group. • Supplementation in those who were *n*-3 PUFA deficient did not lead to improvements in inhibition, working memory, reading, or mean reaction time.
4) Omega-3 PUFA supplementation will improve cognitive performance in ADHD+RND/TD group in studies that met strict inclusion criteria or were of high quality.	No	• Supplementation did not improve cognitive performance in these subgroups.
5) Omega-3 PUFA supplementation will improve cognitive performance in ADHD+RND/TD group in studies that supplemented with adequate EPA or included participants with more homogenous cognitive impairments.	No	• Supplementation did not improve cognitive performance in these subgroups.
Chapter 3: The effect of omega-3 polyunsaturated fatty acid supplementation on emotional lability, oppositional behaviour and conduct problems in ADHD: A systematic review and meta-analysis.		
Primary hypothesis		
6) Omega-3 PUFA supplementation will improve EL, oppositional behaviour, conduct problems, and aggression in children with ADHD or with a related disorder (e.g. disruptive behaviour disorder)	No	• Supplementation did not lead to improvements in EL and related domains of oppositional behaviour, conduct problems and aggression in children with ADHD+RND.

Hypothesis	Supported?	Specific Results
(ADHD+RND group).		
Secondary hypothesis (subgroup analysis)		
7) Omega-3 PUFA supplementation will improve EL and oppositional behaviour in children with ADHD+RND in studies that met strict inclusion criteria or were of high quality.	Partially	• In studies that met strict inclusion criteria supplementation was found to have a small effect on EL. There was a trend for an effect on oppositional behaviour. • In high quality studies there was an indication of an effect on oppositional behaviour. No effects were found for EL.
8) Omega-3 PUFA supplementation will improve EL, oppositional behaviour and aggression in children with ADHD or with a related disorder in studies that included participants with elevated levels of EL or supplemented with adequate EPA.	Partially	• In studies that supplemented with adequate EPA there was a trend for supplementation to improve oppositional behaviour. There was no effect on aggression or EL. • In studies that supplemented children with elevated levels of EL there was no effect on oppositional behaviour, no other domains could be examined.
Chapter 5: The OCEAN study: A randomised controlled trial of omega-3 supplementation in adults with ADHD.		
9) Compared to controls, adults with ADHD will show impaired cognitive performance and increased symptoms of emotional lability (as well as ADHD symptoms).	Yes	• Adults with ADHD compared to controls had impaired cognitive task performance and significantly higher levels of ADHD symptoms and emotional lability.
10) Adults with ADHD compared to controls will have reduced blood *n*-3 PUFA levels and a higher *n*-6:*n*-3 PUFA ratio.	No	• Adults with ADHD did not have reduced *n*-3 PUFA or a higher *n*-6:*n*-3 ratio compared with controls. • In contrary to the hypothesis adults with ADHD compared with controls had *higher* levels of the *n*-3 PUFA DPA.
11) Supplementation with *n*-3 PUFA in adults with ADHD will improve cognition.	No	• Supplementation did not improve cognitive performance in either the ITT or per-protocol analysis.
12) Supplementation with *n*-3 PUFA in adults with ADHD will improve ADHD symptoms and EL.	Weak evidence	• Supplementation had no effect on ADHD symptoms or emotional lability in the ITT analysis. • Supplementation showed a nominally significant improvement in inattention and a trend for improvement in EL.
Chapter 6: The effects of Sativex on neurocognitive and behavioural function in adults with attention-deficit/hyperactivity disorder: The EMA-C study (Experimental Medicine in ADHD - Cannabinoids)		
13) Treatment with Sativex in adults with ADHD will improve cognitive performance.	Partially	• Treatment resulted in a trend for improved cognitive performance in the ITT and per-protocol analysis. • Results interpreted in light of potential bias from an increased drop-out rate in

Hypothesis	Supported?	Specific Results
		the placebo group
14) Treatment with Sativex in adults with ADHD will improve symptoms of ADHD and emotional lability.	Partially	• Treatment resulted in a nominally significant improvement in hyperactivity/impulsivity and a trend for improvement in inattention in both the ITT and per-protocol analysis. • Treatment resulted in a trend for improvement in EL in both the ITT and per-protocol analysis (albeit evidence for improvement was weaker than for symptoms of ADHD).

Note. ITT = Intent-To-Treat; EL = Emotional lability

Chapters 2 and 3 examined the effect of *n*-3 PUFA supplementation on cognitive performance (Cooper et al., 2015) and EL (and related domains of oppositional behaviour, conduct problems and aggression) (R E Cooper et al., 2016) using a systematic review and meta-analysis. Effects on EL and cognitive performance were investigated in children with ADHD (or who had a related/overlapping neurodevelopmental disorder such as dyslexia or disruptive behavioural disorder (ADHD+RND group). This was on the basis that meta-analyses have found a small to moderate effect of *n*-3 PUFA supplementation on reducing symptoms of ADHD, however no study has yet summarised effects on the associated impairments of cognition and EL. We further included typically developing children and adults (TD group) in the cognition study. This was on the basis that, despite limited evidence, omega-3 products are often promoted as cognitive enhancers and are widely consumed in the general population. Results were as follows:

Chapter 2: The effect of *n*-3 PUFA supplementation on cognitive performance in a TD and ADHD+RND group was examined. Cognitive performance was classed into nine domains: IQ, inhibition, attention (measured with omission errors), working memory, short-term memory, reading, spelling, mean reaction time and reaction time variability. Twenty four studies (14 in TD children and adults and 10 in ADHD+RND children) were included in the quantitative synthesis. Results showed no effect of *n*-3 PUFA supplementation in either the whole sample, or the TD or ADHD+RND group when analysed separately. The only significant outcome was for a small improvement in short-term memory in those who may have been deficient in *n*-3 PUFA (SMD=0.3; across the TD and ADHD+RND groups). It was concluded that future treatment trials should consider recruiting only participants who are *n*-3 PUFA deficient. It was further concluded that regulators and producers of *n*-3 products should take this evidence, of a generally null effect on cognition, into account when promoting their products.

Chapter 3: The effect of *n*-3 PUFA supplementation on emotional lability, oppositional behaviour, conduct problems, and aggression in an ADHD+RND group was investigated. Ten studies in children were included in the quantitative synthesis. Although the initial analysis in the whole sample found no significant treatment effects, subgroup analyses suggested small effects (SMD=0.15-0.25) could be present. In studies that met strict inclusion criteria a small but significant effect of supplementation was found for EL and a trend for an effect on oppositional

behaviour. In high quality studies a nominally significant effect on oppositional behaviour was found. In studies that supplemented with adequate EPA there was a trend for an improvement in oppositional behaviour. It was concluded that small effects of *n*-3 PUFA supplementation on EL and oppositional behaviour are possible, however, given the size of these effects, *n*-3 PUFA may not be appropriate as a monotherapy for reducing these symptoms. Future studies in larger samples are required to clarify effects.

Results from these reviews showed that there are as yet no published trials of the effect of *n*-3 PUFA supplementation in adults with ADHD. The following chapter therefore presented results from an RCT of *n*-3 PUFA supplementation in adults with ADHD.

Chapter 5: This chapter presented results from a case/control comparison and RCT of *n*-3 PUFA supplementation in 81 adults with ADHD and 30 typically developing controls. Baseline comparisons showed the ADHD cases to have significantly impaired cognitive performance and more severe symptoms of EL (as well as ADHD) compared to controls. We also found two novel results. First, we found evidence for sensitivity to reward and presentation rate of stimuli during a reaction time task, the Fast Task, for adults with ADHD. This has previously only been found in children with ADHD (Andreou et al., 2007; Cheung et al., under review.; Slusarek et al., 2001; Uebel et al., 2010), and therefore suggests developmental stability of this trait. Sensitivity to rewards is consistent with the 'state regulation' hypothesis of ADHD which proposes cognitive deficits in ADHD are the result of a reduced energetic state and are therefore malleable in the presence of rewards or a faster event rate (this will be discussed in more detail in section 7.4.5) (Kuntsi et al., 2012; Sergeant, 2000). We have therefore provided evidence to suggest the persistence of deficient state regulation from childhood to adulthood in ADHD. The second novel finding was objective evidence under task conditions of difficulties with emotion regulation/over-reactivity and reduced frustration tolerance in adults with ADHD, during a task designed to induce frustration. Baseline comparisons then showed there to be no difference in the blood *n*-3 PUFA levels of adults with ADHD compared with controls that might potentially have contributed to the observed case-control differences as suggested by some authors. On the contrary, there was an indication of slightly higher *n*-3 PUFA levels (for docosapentaenoic acid (DPA)) in the ADHD group. Results from the RCT showed no indication of a treatment effect on cognition, ADHD symptoms, or EL in the intent-to-

treat (ITT) analysis, but marginal evidence for improvement in inattention and, to a lesser extent EL, in the per-protocol analysis. These results should be interpreted cautiously in light of limitations due to a high drop-out rate. They provide only very limited evidence for an effect of *n*-3 PUFA on inattention and potentially EL in adults with ADHD, with any possible effects likely to be small.

Chapter 6: This chapter presented results from an RCT of the cannabinoid medication Sativex Oromucosal Spray in 30 adults with ADHD. Sativex was found to lead to nominally significant improvements in hyperactivity/impulsivity and a trend for improvement in inattention. There were further indications for improvement in cognition for commission errors and the coefficient of variation (CV). While these effects on cognition were non-significant in this study, the effect was a positive one and in the opposite direction to previous findings of impaired cognitive performance in non-ADHD subjects. A sensitivity analysis suggested however that these results might have been biased towards a treatment effect due to an increased drop-out rate in the placebo group. As a whole, given the small sample size and potentially moderate to large effects, this initial evidence is promising. Further investigations into the role of cannabinoids in the treatment of ADHD symptoms and associated impairments, and the underlying mechanisms, is therefore warranted on the basis of these results.

7.3 How results relate to each other

7.3.1 Differences in the treatment response of cognitive and behavioural symptoms

One recurrent theme that has been supported in all four chapters is that of a greater response of the behavioural rather than cognitive symptoms to treatment in adults and children with ADHD. Chapter 2 found no effect of *n*-3 PUFA supplementation on cognitive performance in children with ADHD, while Chapter 3 found preliminary evidence that supplementation may have a small effect on reducing symptoms of EL and oppositional behaviour. Chapter 5 followed this pattern: finding no indication for an effect of *n*-3 PUFA supplementation on cognitive performance, but some preliminary evidence for an improvement in inattention and to a lesser extent EL. Finally Chapter 6 followed a similar pattern with stronger treatment effects for ADHD symptoms (and potentially EL) than cognitive performance. Differential response of the behavioural and cognitive symptoms to treatment is in line with previous meta-analyses and systematic reviews, which have found a

smaller treatment effect of stimulant medication on cognitive performance (d=~0.2-0.6) (Coghill et al., 2014) than on ADHD symptoms (~0.8-1.0) (Banaschewski et al., 2006; Faraone & Buitelaar, 2010). It has also been observed that during the clinical response to methylphenidate there is a dissociation of the treatment effects on ADHD symptoms and cognitive performance in children and adolescents with ADHD (Bédard et al., 2014; Coghill et al., 2007; K. P. Schulz et al., 2014). This, and the evidence of differential response to treatment in this study, supports the proposal that different mechanisms could be responsible for change in cognitive performance and change in behavioural symptoms (Coghill et al., 2007).

Differences in treatment response of behavioural symptoms and cognitive performance may reflect the neuropsychological heterogeneity typically seen in ADHD. This is on the basis that the ADHD diagnosis is made on behavioural not cognitive symptoms and therefore the participants included in this thesis, whilst being relatively homogenous for symptoms of inattention and hyperactivity/impulsivity, will be more heterogeneous for cognitive deficits and symptoms of EL. This is illustrated by the effect sizes of the case/control differences with the largest being for ADHD symptoms (the selection variable): d=2.8-3.7, then rating scale measures of EL d=1.9-2.3 and the smallest for cognitive performance: d=0.3-1.0. Reduced baseline impairments in cognitive performance will lead to a reduced effect size from treatment. Future work, which has cognitive performance as the primary outcome, could screen participants for deficits in these measures and recruit those who are above a certain threshold of impairment. This would allow for better investigation of treatment effects on cognition.

7.3.2 Sativex and *n*-3 PUFA supplementation as a treatment for adults with ADHD

This thesis has examined two potential alternative treatments for ADHD; *n*-3 PUFA supplementation, and Sativex Oromucosal Spray. Results from the RCT indicate that Sativex has more potential as treatment than *n*-3 PUFA supplementation. Sativex resulted in a trend for improvement in cognitive performance (at a moderate effect: d=~0.5-0.6) whereas *n*-3 PUFA supplementation showed no effect on this measure. Sativex, in *both* the ITT and per-protocol analysis, showed a nominally significant effect of improving symptoms of hyperactivity/impulsivity (at large effect: d=~0.9-1.00) and a trend for improving inattention (at moderate effect: d=~0.6-0.7) whereas *n*-3 PUFA supplementation showed a nominally significant effect on improving

inattention in the per protocol analysis *only* (although the moderate effect size was equivalent to that found for Sativex: d=0.7). Both treatments showed only trends for improvements in EL, although for Sativex this was found in *both* the per-protocol and intent-to-treat (at moderate effect: d=∼0.5-0.6), whereas for *n*-3 PUFA supplementation this was only in the per-protocol (although again, the moderate effect sizes were equivalent: d=∼0.5). Therefore as a whole, results from this thesis give preliminary evidence for a moderate effect of *n*-3 PUFA supplementation on reducing EL and inattention but stronger evidence for moderate to large effects of Sativex on improving ADHD symptoms, EL, and to a lesser extent cognitive impairments in adults with ADHD. An RCT of Sativex in a larger sample (150-200 people: based on power calculations in the discussion of Chapter 6 (section 6.6)) will be required to confirm these promising results.

7.3.3 Mechanism of action of *n*-3 PUFA and Sativex

One of the theories behind the mechanism of action of the two treatments researched in this thesis is that they act through the dopamine system. Through alterations of cellular communication, deficiencies in *n*-3 PUFA have been linked to altered neurotransmission including dopamine (Assisi et al., 2006; Chalon, 2006; Haag, 2003; Young & Conquer, 2005). A number of studies in animals and humans have found administration of the cannabinoid Δ9-THC to increase dopamine in the striatum (Bossong et al., 2009, 2015; Kuepper et al., 2013) and in other brain areas implicated in ADHD (Chen et al., 1990; Pistis et al., 2002; Stokes et al., 2010). Given that a common theory of ADHD is that of dopamine abnormalities (del Campo et al., 2012; Hesse et al., 2009; Krause et al., 2005; Krause et al., 2000), and that this thesis has given promising results for Sativex as a treatment for adult ADHD, preliminary evidence for *n*-3 PUFA supplementation as a treatment in children with ADHD, and to a lesser extent adults, we have provided some evidence to warrant further investigation of the mechanisms of action of *n*-3 PUFA and Sativex, to ascertain whether any improvements in symptoms and cognition are mediated by changes in dopamine.

7.4 How results relate to other research findings

7.4.1 The effect of Omega-3 PUFA on cognition and emotional lability

Results from Chapter 2, of there being no evidence for an effect of *n*-3 PUFA supplementation on cognition in TD children, and adults and children with ADHD+RND, is in agreement with the mixed evidence from individual studies (Antypa, Van der Does, Smelt, & Rogers, 2009; Dalton et al., 2009; Hirayama et al., 2004; Jackson, Deary, Reay, Scholey, & Kennedy, 2012; Parletta, Cooper, Gent, Petkov, & O'Dea, 2013; Sinn et al., 2008; Vaisman et al., 2008; Voigt et al., 2001). These results go against narrative reviews which have suggested *n*-3 PUFA to improve cognitive performance (Assisi et al., 2006; Bryan et al., 2004; Horrocks & Yeo, 1999; Stonehouse, 2014). However these reviews failed to provide a critical examination of the literature and, as they were narrative and therefore did not include meta-analyses, were limited in their ability to provide firm conclusions.

Results from Chapter 3 provide evidence for small effects of *n*-3 PUFA on EL and oppositional behaviour in children with ADHD+RND. This is in line with epidemiological and cross-sectional studies which have associated deficiencies in *n*-3 PUFA with emotionally labile behaviour in children adolescents and adults with and without ADHD (Corrigan et al., 1994; Gow et al., 2013; Hibbeln, 2001; Iribarren et al., 2004; Stevens et al., 1996). Results are also in line with a meta-analysis which found *n*-3 PUFA supplementation to lead to significant reductions in aggression in children, adolescents, and adults with and without ADHD (Benton, 2007). This meta-analysis found a larger effect size (SMD =0.61) than was found in Chapter 3 (SMD=0.15-0.25) which was attributed to a number of limitations to the study by Benton, including the combining of a range of heterogeneous measures (e.g. psychological tasks with rating scales), and study populations (e.g. ADHD and Borderline Personality Disorder).

Taken as a whole, results from Chapters 2 and 3 (Cooper et al., 2015, 2016), combined with previous meta-analyses (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), mean that we now have the most accurate idea possible of the effects of *n*-3 PUFA supplementation on ADHD symptoms and associated impairments in cognitive performance. Omega-3 PUFA supplementation, in children with ADHD appears to have a small but significant effect on reducing symptoms of ADHD (SMD=0.21-0.31), and to a lesser extent EL and oppositional behaviour (SMD=0.15-0.25). Supplementation does not appear to affect cognitive performance aside from

potentially in children with ADHD who are deficient in *n*-3 PUFA (SMD=0.26). Effects on behaviour have been proposed to be mediated through alterations in neurotransmitters, particularly dopamine, a neurotransmitter which has been implicated in ADHD (Bolea-Alamañac et al., 2014), although there is currently no direct evidence to support this. Therefore *n*-3 PUFA supplementation could be used as a treatment for ADHD in children, although given the small effect sizes in comparison to pharmacological treatment it is advised that it be used as augmentation to traditional treatment, or as a monotherapy for those who do not wish to use pharmacological treatment.

7.4.2 Omega-3 PUFA as a treatment for adults with ADHD

The finding of no difference, or even potentially higher, levels of *n*-3 PUFA in adults with ADHD compared with controls (Chapter 5) goes against a recent meta-analysis (Hawkey & Nigg, 2014) and individual studies (Chen et al., 2004; Stevens et al., 1995; Germano et al., 2007; Antalis et al., 2006). These studies have found reduced blood levels of *n*-3 PUFA (particularly EPA and DHA) and an increased *n*-6:*n*-3 PUFA ratio in children and adults with ADHD compared to controls. Although on closer examination of the meta-analysis the majority (6/9) of included studies were conducted in children. The effect sizes from studies in children were also generally more significant than those conducted in adults. Therefore it is possible that *n*-3 PUFA differences may be more apparent in children than in adults. This weakens the proposed causal relationship between *n*-3 PUFA levels and symptoms of ADHD in adults, although this may still hold true in children. Large cross-sectional studies examining case/control differences in *n*-3 PUFA levels and longitudinal studies examining *n*-3 PUFA levels and ADHD symptoms from childhood to adulthood are required to clarify the role of *n*-3 PUFA in ADHD; although the previously reported treatment trials in children do suggest they play some role.

Supplementation with *n*-3 PUFA gave weak evidence of improvements in inattention and potentially EL, and no evidence for improvements in cognition. As previously discussed this is in line with meta-analyses conducted by myself and other research groups which have found small effects of supplementation on ADHD symptoms in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013), and to a lesser extent EL (Cooper et al., 2016), and no effect of supplementation on cognitive performance (Cooper et al., 2015). To our knowledge this is the

first study in adults with ADHD. Our conclusions of weak evidence for an effect of supplementation on inattention and EL were limited by a high drop-out rate and low power and as such no firm conclusions can be drawn. We can only exclude large effects and it may still be the case that smaller effects, similar to those seen in children, may emerge. A further study in a larger sample is therefore warranted. In line with this, a second RCT in 60 adults with ADHD is currently being conducted by Michael Rösler's group at Saarland University (Germany). This group have used the same outcome measures for ADHD symptoms (CAARS/WRADDS (Conners et al., 1999; Wender, 1995)) and EL (CNS-LS (Moore et al., 1997)/ALS (Oliver & Simons, 2004)) as those used in the current study, and we will be combining our data in the near future.

7.4.3 Sativex as a treatment for adults with ADHD

Our study of the effects of the cannabinoid medication Sativex in adults with ADHD, is to our knowledge, the first of its kind, and therefore direct comparisons to previous research studies cannot be made. However the subjective accounts of patients which provided the impetus for this study suggested symptom improvement might be substantial and perhaps similar to that found in response to traditional ADHD medications. This was supported by the effect sizes found in this study of d=0.7-1.0 for ADHD symptoms, and d=0.5-0.7 for cognition, which are similar to the effect sizes found on these domains for stimulant medication (Coghill et al., 2014; Faraone & Buitelaar, 2010; Faraone & Glatt, 2010; Faraone et al., 2004; Mészáros et al., 2009). This study has therefore provided initial evidence to support the 'self-medication' hypothesis in ADHD. This hypothesis suggests that the high rates of substance abuse found in ADHD is due to patients attempting to 'self-treat' with substances such as stimulants or cannabis (Bolea-Alamañac et al., 2014; Horner & Scheibe, 1997; Wilens, 2004). In light of evidence that heavy cannabis users show a more positive response to cannabis than occasional users it was suggested that those who are 'drawn' to cannabis could be 'innately' protected from the negative effects of the drug (D'Souza et al., 2008; Ramaekers et al., 2009). Given the high incidence of cannabis use in adults with ADHD, it is possible those with ADHD represent a subgroup of individuals who respond more positively to this drug.

7.4.4 Emotional lability and cognitive performance in adults with ADHD

Case/control differences in symptoms of ADHD, EL, and cognitive performance found in adults with ADHD (Chapter 5) are supported by numerous research results. This is highlighted best by the DSM 5 diagnostic criteria which, along with elevated symptoms of hyperactivity/impulsivity and inattention, also lists EL and cognitive impairment as associated features that support the diagnosis of ADHD (American Psychiatric Association, 2013). This investigation also gave two novel results in adult ADHD relating to this.

7.4.5 Reward sensitivity in adults with ADHD

The first novel result, found evidence of reward/presentation rate sensitive improvements in cognitive performance on the Fast Task. This is the first time this has been found in an adult ADHD sample, and is supported by repeated evidence of the same association in children with ADHD (Andreou et al., 2007; Cheung et al., under review; Slusarek et al., 2001; Uebel et al., 2010). Furthermore, the recent finding that such sensitivity to reward and presentation rate was not present in a sample of children with ASD (Tye et al., under review) suggests this to be a developmentally stable deficit with some specificity to ADHD. This finding is in support of the state regulation hypothesis of ADHD (Sergeant, 2000). This model proposes deficits in cognition to be the result of a reduced energetic state. Reward sensitivity in ADHD highlights the malleability of RTV (Kuntsi et al., 2012); supporting the advantages of incorporating fast-paced activities and incentives into the environment of children and adults with ADHD, in order for them to function at an optimal level (Cheung et al., under review). From a theoretical perspective this finding indicates a potential role of state regulation deficits in the symptoms and impairments of ADHD across the lifespan. From a clinical perspective, observations of ADHD patients included in the studies in this thesis and more generally (Asherson, personal communication) show that patients with ADHD can function well in stimulating environments or situations. For example adults with ADHD often do well in entrepreneurial professions or as successful sports persons. The beneficial effects of exercise in ADHD is a growing but important area of research (Rommel et al., 2013; Rommel et al., 2015). Furthermore, I have been involved in a qualitative research project during this PhD which has asked adults with ADHD about the positive aspects of their diagnosis. It is important to understand the optimum ways in which ADHD can be managed, in order to give the best advice clinically to help improve function.

7.4.6 Emotional overreactivity in adults with ADHD

The second novel result was that of objective evidence of difficulties in emotion regulation/overreactivity and reduced frustration tolerance in adults with ADHD in response to a frustration task. Post-task ratings of irritability and frustration also mapped onto more subjective rating scale measures of EL. This is, to our knowledge, the first study to examine response to a frustration task in adults with ADHD during experimental task conditions. Results are in agreement with findings in children with ADHD which have found deficiencies in emotion regulation and reduced frustration tolerance in response to a 'frustrating' puzzle-task (Martel, 2009; Melnick & Hinshaw, 2000; Scime & Norvilitis, 2006; Walcott & Landau, 2004). Results are also in agreement with a previous 'real world' study using experience sampling that found emotional overreactivity and instability in adults with ADHD in response to perceived 'bad events' (Skirrow et al., 2014). Our results combined with these previous findings suggest developmental stability of difficulties with emotion regulation and management. The PASAT-C could be used as an objective test for emotional lability in adults with ADHD.

7.5 Strengths and weaknesses

7.5.1 Power

One of the main limitations noted in Chapters 2 and 3 was that the majority of RCTs examining the effects of *n*-3 PUFA supplementation in both TD and ADHD+RND populations were underpowered. In both the cognition and EL meta-analysis, the treatment effects were small (~d=0.3). With this modest effect size a sample size of around 352 participants (β=80%, two-tailed α = 0.05) would be required to confirm or refute these small effects. In Chapter 2, trials in the ADHD+RND group ranged from 40-362 participants and included only one study that was adequately powered. Trials in the TD group ranged from 38-422 participants and included only two studies that were adequately powered. In Chapter 3, trials ranged from 21 to 362 participants and included only one study that was adequately powered. This undermines our ability to provide conclusions of the lack of or presence of treatment effects in Chapters 2 and 3 respectively. Future studies should be adequately powered to detect small effects in order to clarify the presence of treatment effects.

Both the EMA-C and OCEAN studies were underpowered. It was estimated that in order to detect the expected small effect (d=~0.3) of *n*-3 PUFA supplementation on symptoms of ADHD a future RCT in adults with ADHD would require a sample size of 352 participants. For the EMA-C study a sample size of 150-200 participants was recommended for future studies, based on the medium to large effect sizes that were found (d=0.5-0.7). Although lack of power is a significant limitation, as both these studies were exploratory pilot studies it is not unusual for them to be underpowered. The aim of the pilot study is to guide future, larger studies through the examination of study-feasibility, trends in treatment effect, and exact effect sizes for future power/sample size calculations. This has been achieved, with promising effects from Sativex and weaker, albeit potential, effects of *n*-3 PUFA supplementation on ADHD symptoms. Observations from running the studies can also be used to improve future trials, such as the need for a shorter follow-up period and testing sessions in order to attempt to limit drop-out rate and alteration of the dosing guidelines for the EMA-C study.

7.5.2 Effect sizes

The clinical application of results from the OCEAN study are limited by the high drop-out rate, lack of power and small effect sizes. Results gave limited evidence for a small treatment effect of *n*-3 PUFA supplementation on improving symptoms of inattention and EL, with no evidence for effects on cognitive performance (see Section 7.4.2 for a more detailed discussion). This is in line with the meta-analyses conducted in this thesis (Cooper et al., 2016; Cooper et al., 2015), and previously (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014; Sonuga-Barke et al., 2013). It is also in line with mixed evidence from individual placebo-controlled trials which have examined the effects of *n*-3 PUFA supplementation on ADHD symptoms (Sinn & Bryan, 2007; Voigt et al., 2001), cognitive performance (e.g. Milte et al., 2012;Sinn et al., 2008), and EL (Stevens et al., 2003; Widenhorn-Müller et al., 2014). Given that potential treatment effects are likely to be small, the data from this thesis does not suggest that *n*-3 PUFA supplementation could be a clinically relevant treatment for ADHD in adulthood, especially as a monotherapy. Although in light of the high drop-out rate, further, fully powered studies (in ~ 352 adults with ADHD) are required before we can say whether these findings are robust. In order to overcome potential problems from drop-outs leading to reduced power a future study could consider over-recruiting or using a sequential trial design. For example, sequential trials conduct a number of interim analyses and stop recruitment once the trial

is fully powered (in this case once 352 participants have completed the trial) (Sedgwick, 2012). Such a design could ensure that a fully powered study of *n*-3 PUFA supplementation in adults with ADHD is conducted and could further establish whether the indications of small effect sizes found in this thesis are robust.

7.5.3 The role of pilot studies in meta-analyses

Given the work described in this thesis consists of meta-analyses and pilot-RCTs it is important to discuss the link between these two research methods. The meta-analyses in chapters 2 and 3 combined a number of small and large RCTs in order to produce a weighted effect. The majority of studies included in these meta-analyses, similarly to the OCEAN and EMA-C studies, were underpowered to detect the expected small effects of *n*-3 PUFA supplementation on cognitive performance and EL (for further discussion see Chapter 2 (discussion section) and Section 3.5). For example, a number of the studies of *n*-3 PUFA supplementation in children with ADHD had a similar or smaller sample size to the OCEAN study (e.g. Milte et al., 2012; Richardson & Puri, 2002; Stevens et al., 2003; Vaisman et al., 2008; Voigt et al., 2001). Therefore pilot or feasibility studies such as the OCEAN and EMA-C studies reported in this thesis can be included in meta-analyses as they provide some indication of trends in treatment effect. However one limitation that must be taken into account when including underpowered studies in meta-analyses is that combining these studies does not give the same power that one fully powered study would have. For example the meta-analysis in Chapter 3 found a small treatment effect of *n*-3 PUFA supplementation on EL across four studies which included a total sample of 515 participants. Although this was found in a large total sample this is not equivalent to or as robust as the same finding in *one* study of 515 participants. Therefore meta-analysing a number of smaller studies does not make up for lack of power. Once initial pilot or feasibility studies have been conducted researchers must aim to conduct large fully powered studies to gain the most reliable estimates of treatment effect.

7.5.4 Drop-out rate

A limitation to both of the RCTs (Chapters 5 and 6) is that of small sample size and loss to follow-up. In the OCEAN study (Chapter 5) we experienced a high drop-out rate across both the placebo and active groups which will have considerably reduced power. The reasons for drop-outs were similar across both groups, and there was no significant difference in number of drop-outs between the

active and placebo groups. Drop-outs were therefore presumed to be at random which limits potential bias caused by drop-out. Reasons for drop-outs and measures to avoid this in future studies are detailed below in Section 7.6.2.

In the EMA-C study (Chapter 6), a higher drop-out rate was observed in the placebo than the active group. Given the failure of the blinding (since participants were able to correctly guess which arm they were in) and observations from running the study, this drop-out may have been non-random due to the relative lack of any effect of the placebo. Although data imputation under the missing not at random (MNAR) and missing at random (MAR) assumption gave similar results, this is likely due to the small number of drop-outs. A future larger study could consider recruiting a higher number of participants into the placebo group to try and limit any bias caused by non-random drop-out.

7.5.5 Heterogeneity of EL and cognitive deficits in ADHD

Another limitation noted in both Chapters 2 and 3 is that the participants included in the various studies will have been heterogeneous with regard to EL and related behavioural domains and also cognitive impairments. This heterogeneity will lead to a reduced treatment effect in comparison to ADHD symptoms where there is a more uniform deficit (Coghill et al., 2007). Future studies, especially where the primary outcome is EL or cognitive performance, should ensure a certain threshold of impairments in these domains when recruiting participants.

7.5.6 Blinding

The EMA-C study was limited by the difficulties in blinding the medication due to the obvious effects of Sativex to most participants. Blinding in the OCEAN study was, however, more successful. The failure of the blind in the EMA-C study is a problem inherent to the use of a cannabinoid medication, the effects of which appear to be apparent to many participants. Lack of blinding is associated with exaggerated estimates of treatment effect (Pildal et al., 2007), in particular for subjective outcome measures (Wood et al., 2008). Failure of the blind could therefore have biased the behavioural outcomes (such as ADHD symptoms) more so than the objective outcomes (cognitive performance). Given the failure of the blind and also non-random drop-out, potential exaggeration of treatment effects must be taken into account when interpreting results. Future

trials should compare Sativex to current ADHD stimulant medication to reduce the chance of breaking the blind.

7.5.7 Concomitant medication

The use of concomitant medication (e.g. stimulants, antidepressants) in the OCEAN study was a limitation. Concomitant medication may have reduced the severity of ADHD symptoms and associated cognitive deficits leading to reduced effect sizes for the effect of treatment with *n*-3 PUFA. This may have been especially so for participants taking non-stimulant or antidepressant medication, as they could not come off this medication for the testing sessions (nb. those on stimulant medication stopped taking this for 48 hours before testing sessions). Therefore one of the strengths of the EMA-C study was that the participants were unmedicated for a week before and throughout the 6 week trial. This meant that we could effectively investigate the effects of Sativex as a monotherapy in the presence of high levels of ADHD symptoms.

7.5.8 Dosage

One strength of the OCEAN study was the use of a high EPA dose *n*-3 PUFA supplement. The supplementation (Equazen High Concentrated: 1,116 mg EPA, 348mg DHA, and 120mg GLA per day) was high in EPA and taken at a dose which was at the upper limit of recommended daily *n*-3 PUFA intake (~1500mg per day) (Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006; Molendi-Coste, Legry, & Leclercq, 2011). Previous meta-analyses have found EPA to have greater efficacy in reducing ADHD symptoms in children (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014) and advised the use of high EPA supplementation in future trials (Bloch & Qawasmi, 2011). Therefore a strength of the OCEAN study is that, based on previous evidence, the optimum doage of *n*-3 PUFA for ADHD treatment was used.

One weakness however of the OCEAN study is that the evidence for dosage is based on studies in children with ADHD not adults (Bloch & Qawasmi, 2011; Hawkey & Nigg, 2014). The OCEAN study was the first trial conducted in adults with ADHD. Although a high EPA supplement has been found to be efficacious in children, this may not be the case in adults whose brains may no longer be developing. Furthermore whilst previous research has found children and adults with ADHD to have reduced blood levels of *n*-3 PUFA (particularly EPA and DHA) (Hawkey & Nigg, 2014), this was

not the case for the sample included in the OCEAN study who appeared to have similar, if not slightly higher, levels of blood *n*-3 PUFA than controls (see Section 5.6.2). Closer examination of the adult studies included in the meta-analysis (which showed reduced *n*-3 PUFA levels in ADHD) suggests that had this analysis been conducted separately for children and adults, evidence for *n*-3 PUFA deficiencies in adults may have been weaker than that in children (Hawkey & Nigg, 2014). Evidence from this thesis therefore suggests that different dosages of *n*-3 PUFA supplementation could be required in children and adults. In order to clarify the required dose, future large cross-sectional case/control comparison studies, and subsequent meta-analyses need to be conducted in order to establish whether an *n*-3 PUFA deficiency is present in adults. If this is established suitable doses of *n*-3 PUFA need be developed and trialled in future, adequately powered (n=~352) trials.

Dose was also a potential limitation in the EMA-C study where the optimum dose of Sativex may not have been used by every participant (see Section 6.6 for a more detailed discussion). Given this study was, to our knowledge, the first to examine Sativex as a treatment for ADHD, the dose at the start of the trial was unknown. The titration schedule we used was provided by GW Pharma and was for symptom relief in patients with multiple sclerosis. It became clear early on in the trial that this titration schedule was unsuitable for adults with ADHD: it increased too quickly, to too high a dose (see Section 6.4.5.2). Although patients were verbally informed (at the baseline testing session and during regular telephone monitoring) to remain at a lower dose if they were finding effects from the medication, at follow-up a small number of participants had taken a high dose for the majority of the trial, reporting that they had not derived benefits. Failure to maintain an optimal dose in each participant may have reduced the treatment effect in the active group. It is important that a new titration schedule (which increases to a maxmum dose of 8-9 sprays as compared to 14 in the EMA-C study) be developed for a future trial in order to clarify the effect of Sativex in adults with ADHD when taken at a more optimal (lower) dose.

7.5.9 Blood *n*-3 PUFA levels in adults with ADHD

As discussed above (Section 7.5.88), the sample of adults with ADHD included in the OCEAN study had similar if not slightly higher levels of blood *n*-3 PUFA than controls. This went against hypothesis (see section 5.2.1) and previous research (Antalis et al., 2006; Chen et al., 2004; Germano et al., 2007; Hawkey & Nigg, 2014; Stevens et al., 1995). This lack of *n*-3 PUFA deficiency

is a limitation for the OCEAN study as it may have reduced the chance of observing a treatment effect following supplementation with *n*-3 PUFA. For example, the meta-analysis in Chapter 2 found the only evidence for effects of supplementation to be in those who were *n*-3 PUFA deficient (albeit at small effect) (Cooper et al., 2015).

Closer examination of previous research suggests evidence for *n*-3 PUFA deficiencies in adults with ADHD may be weaker than that in children with ADHD (Hawkey & Nigg, 2014). Furthermore the case/control sample included in the OCEAN study appears to be the largest sample to date to investigate *n*-3 PUFA differences in adults with ADHD. Therefore evidence from this thesis could suggest that there may not be *n*-3 PUFA differences in adults with ADHD compared to controls. This could question the proposed causal relationship between *n*-3 PUFA and symptoms of ADHD. For example it is suggested that *n*-3 PUFA may be linked to dopamine neurotransmission and in turn the symptoms of ADHD (see Section 1.2.5.3). Evidence from this thesis undermines this causal hypothesis. Instead it could be the case that *n*-3 PUFA deficiencies are one factor that contributes towards ADHD symptom severity in childhood. However given that some but not all (~60%) of ADHD cases persist into adulthood (Faraone et al., 2006), *n*-3 PUFA deficiency may not be one of the factors that contributes to adult ADHD. A more controversial explanation is the recent suggestion that at least in some cases adult ADHD may be a different disorder from childhood-onset forms of the condition (Moffitt et al., 2015). Future work would be required to test these potential hypotheses.

7.5.10 Treatment duration

Treatment duration differed greatly between the two trials. The OCEAN study was a relatively long-term study with follow-up at 6-months whilst the EMA-C study was a short-term study with follow-up at 6 weeks. Although a long follow up was chosen for the OCEAN study, evidence from studies in children with ADHD suggests that treatment effects may be uninfluenced by study duration (Bloch & Qawasmi, 2011; Cooper et al., 2016; Cooper et al., 2015). In Chapters 2 (Cooper et al., 2015) and 3 (Cooper et al., 2016) we found no relationship between length of supplementation with *n*-3 PUFA and treatment effects on EL and oppositional behaviour (in children with ADHD), and on cognitive performance (in children with ADHD and typically developing children and adults), in trials that ranged from 4-52 weeks. This is in line with a meta-analysis which found no relationship between

trial duration and efficacy of *n*-3 PUFA in reducing ADHD symptoms in children (in trials that ranged from 4 weeks-4 months) (Bloch & Qawasmi, 2011). Given the failure to find a relationship between length of trial and treatment efficacy, and the suggestion that the long length of the trial could have contributed towards the high drop-out rate (see Section 7.6.2), the design of the OCEAN study could have been improved by having a shorter follow-up period. Although it is important to note that this recommendation is limited as it is based mainly on evidence from trials in *children* with ADHD, not adults. Although future studies in adults should consider employing a shorter (~3-4 month) follow-up period, more trials of varying length in adults with ADHD may be required to confirm whether efficacy (or lack of efficacy) of *n*-3 PUFA is uninfluenced by trial duration.

In contrast the EMA-C study employed a short follow-up period (6 weeks). Given this was the first study to examine the effects of Sativex in adults with ADHD, the follow-up period was based on previous RCTs of Sativex in patients with multiple sclerosis, or who had neuropathic pain, which found efficacy of Sativex after 5-6 weeks (Collin, Davies, Mutiboko, & Ratcliffe, 2007; Nurmikko et al., 2007). Although this short follow-up period may have contributed to the low drop-out rate, I think the trial could have benefitted from a longer follow-up period (~ 3 months). This is because it would often take participants longer than the 2-week titration period to adjust to taking the Sativex and find an optimum dose of the medication. I feel that the trial was therefore not long enough for participants to stabilise on the medication which may have led to an underestimation of the treatment effect.

7.5.11 Adverse events

A potential limitation to the use of Sativex in adults with ADHD is the number of minor (n=3) and major (n=1) adverse events that were experienced by participants in the active group. In comparison very few (n=3) minor adverse events were experienced by participants in the active, *n*-3 PUFA group. Therefore *n*-3 PUFA supplementation was better tolerated than treatment with Sativex. The number of adverse events experienced in the EMA-C study, particularly at higher doses, could potentially limit the use of Sativex as a treatment for adults with ADHD. As discussed above (Section 7.5.8) it was realised after the first 2-3 participants had begun the trial that the titration schedule provided by GW Pharma was too high. Although participants were verbally informed to not increase the dose too quickly, a small number of particiants may have taken too

high a dose during the trial or 'double-dosed' which was not advised. This may have led to an overestimation of the adverse effects of Sativex. As discussed above, the development of a new titration schedule (which increases to a maxmum dose of 8-9 sprays) is important, in order to clarify the commonality of adverse effects of Sativex when it is taken at a lower dose.

7.5.12 Strengths of the meta-analytic and RCT methods

The meta-analyses conducted in this thesis offer the most precise estimate of the treatment effect of *n*-3 PUFA on cognitive performance and EL to date. The studies were conducted to recommended standards for systematic reviews and meta-analysis. We were careful to combine homogenous measures by, for example, separating parent and teacher ratings of EL in Chapter 3, including only omission errors as a cognitive measure of 'attention' in Chapter 2, and conducting separate analyses in children and adults (in the TD group) in Chapter 2. The low levels of heterogeneity in both studies illustrates the similarity of the combined outcome measures; increasing the reliability of the reported findings.

The strength of our placebo-controlled RCTs is that they are considered the 'gold standard' for the evaluation of treatment efficacy. Although we were limited in our conclusions due to lack of power, the overriding aim of this thesis was to attempt to improve treatments for adults with ADHD by investigating possible alternative treatments and highlighting the potential role of alternative mechanisms for further investigation. I feel this has been met, with cannabinoid medication in particular proving to be a worthwhile area for future research. Furthermore these treatment trials received an extremely positive response from the patients themselves. For the *n*-3 PUFA research, patients were pleased to see an investigation of a non-pharmacological treatment. For the EMA-C study the response was extremely positive with patients feeling that the research was really engaging and listening to them. This was particularly true for those who had received consistent negative feedback in relation to their cannabis use.

7.6 Observations and conclusions from running the OCEAN and EMA-C studies

7.6.1 Clinical observations of the ADHD samples

Observations from the screened ADHD samples are in line with previous research (Section 1.1.5). ADHD in adulthood is a severe and impairing disorder with significantly high rates of comorbid conditions including autism spectrum disorder (ASD), substance use disorder (SUD), depression, anxiety, bipolar disorder, obsessive compulsive disorder (OCD), and schizophrenia. The most common comorbidity was ASD (either diagnosed or suspected). The co-occurrence of ASD and ADHD is well established. In children the prevalence of ADHD in ASD has been reported to be as high as 28-53% (Simonoff et al., 2008; Sinzig, Walter, & Doepfner, 2009) and 32-43% in adults (Anckarsater, Stahlberg, Larson, & Hakansson, 2006; Hofvander et al., 2009). Despite the high prevalence a concurrent ADHD and ASD diagnosis has only recently been acknowledged by the DSM-5 (American Psychiatric Association, 2013). It is important that clinicians bear in mind the high rates of overlap between ASD and ADHD.

The second most common comorbidity in the OCEAN study were depression and anxiety disorders. Research has found risk of depression to be elevated in ADHD, with 35-50% of adults with ADHD experiencing one or more episode of depression during their lifetime, compared to a 15% risk in the general population (Sobanski, 2006). These high rates of comorbidities led to alteration of the exclusion criteria for the OCEAN study to allow participants on antidepressants (or in one case anti-anxiety medication) to take part due to the very high numbers being treated with such medications. This led to 14 participants being recruited into the study who were taking either antidepressants only, or as a concomitant (with stimulants/non-stimulants) medication. It is important that clinicians bear in mind the high rates of overlap between ADHD and depression/anxiety.

Substance abuse problems were also common in the samples screened for both studies. The most common drug of abuse was cannabis. These high rates of cannabis use in adults with ADHD contribute to the theory that individuals are self-medicating with this drug. This observation during the OCEAN study contributed to the motivation to conduct the EMA-C study.

A main reason for exclusion in the EMA-C study was that potential participants were unwilling to stop taking their usual ADHD (stimulant) medication (12.1%). This highlights that, despite this

thesis examining 'alternative treatments', many adults with ADHD are happy with their current medication and gain significant benefit from it (Banaschewski et al., 2006; NICE, 2008). A number of individuals with ADHD told me that they 'cannot function' without their medication or that going on the medication 'changed their lives'. Some individuals had been on medication since early childhood and therefore felt they depended on it to function.

Finally a number of participants declined to take part in the EMA-C study because they did not wish to take a cannabinoid medication (5.1%). This is understandable given that cannabis is classified as a controlled (illegal) Class B drug. In line with this others did not wish to take part as they felt their work would not approve (4%). A potential concern is the link between cannabis use and psychosis (Henquet et al., 2005; Moore et al., 2007). As discussed in Section 1.3.1, recent research has found sinsemilla ('skunk') (which has high Δ9-THC and low CBD) but not cannabis resin ('hash') (which contains relatively similar amounts of Δ9-THC to CBD) to be related to psychosis (Di Forti et al., 2015). The properties of Sativex are more comparable with 'hash' (containing a 1:1 ratio of Δ9-THC:CBD) and therefore it has a much better safety profile than black market cannabis, which is generally dominated by sinsimella, and has a high THC content. The distinction between Sativex and black market cannabis needs to be made clear if Sativex were to be prescribed to patients. There is also an understandable fear of taking any illegal or controlled drug, although 'speed' or street amphetamine is also an illegal Class B drug closely related to dexamphetamine, a commonly prescribed and effective treatment for ADHD (Faraone & Buitelaar, 2010; NICE, 2008). Therefore the use of medications derived from illegal drugs such as 'cannabis' should not be discounted. Patients should be informed of the distinction between these and 'black market' drugs.

Despite the severity of the disorder, a prominent theme that came up during the recruitment process was the difficulty a large number of individuals had in obtaining a diagnosis. For example, 23% of the EMA-C participants and 12% of the OCEAN participants obtained a 'research diagnosis' of ADHD by entering the study, whereas they were previously undiagnosed because of the significant difficulties that still remain in obtaining a diagnostic assessment for ADHD as an adult. In Section 1.1.11.1, I highlighted the factors thought to contribute to the difficulty in obtaining a diagnosis (Kooij et al., 2010). These factors were supported by the experiences of our participants as follows:

1) There may be unease within some mental health professionals in the identification and treatment of ADHD (Bolea et al., 2012; Singh, 2008). For example, some hold the erroneous view that ADHD is a construct manufactured by the pharmaceutical industry (Goldstein, 2006):

Many people would research the diagnosis of ADHD before going to their GP and ask to be referred for an ADHD assessment. However there seemed to often be an unwillingness to make the referral.

2) Lack of training and services in ADHD:

Misdiagnosis was common, as many patients were initially diagnosed with depression or anxiety disorders, but found treatment with antidepressant medication to be ineffective. There appear to be very few specialist Adult ADHD clinics in the UK. People would often not be referred because there was no clinic in the area that they could attend. Alternatively, patients were referred to clinics huge distances from where they lived and would have to incur the expense and time to travel there. Once referred the waiting lists were very long (6 months to 1 year).

3) Clinicians may be reluctant to widen the already overstretched psychiatric services to include adults with ADHD (Bolea et al., 2012):

Very often the funding for the ADHD diagnostic assessment was declined. In the case of funding decline, patients were often unaware that this had occurred so would be left unsupported for months with no feedback.

Due to these problems, a number of patients were diagnosed privately, at great expense. A number of participants also entered the study in order to obtain a research diagnosis from a recognised group with expertise in adult ADHD. There is therefore considerable room for improvement in the NHS in terms of the diagnosis (and subsequent treatment) of ADHD in adulthood. Improved training and increased funding to the service is required. Improvements in training may also reduce any potential unease that some doctors may have in diagnosing and treating the disorder.

7.6.2 Research observations from running the studies

Due to the nature of the condition, disorganisation, appointment cancellations, and no-shows were common, increasing the difficulty of the research process. It is estimated that around 30-40% of participants for the OCEAN study cancelled or re-arranged their appointments, often at short notice. Participants were also often very difficult to contact. This caused the most difficulty in the EMA-C study where it was of great importance that regular contact was maintained during the titration period, in order to monitor the participant's response to the medication and obtain the optimum dosage. Notably, once contact had been made there was no suggestion that participants were reluctant to take part or did not wish to continue in the study. In future studies using Sativex the importance of the titration phone calls must be stressed.

A small number of participants could not tolerate the cognitive testing sessions. One participant in the OCEAN study could not complete the EEG session and two participants in the EMA-C study could not complete the Qb Test as they found it too difficult to maintain their attention. It is possible that a number of the drop-outs from the OCEAN study may have been because the baseline testing sessions were long and cognitively demanding, which may have caused unwillingness for participants to return. This may have been compounded by the long follow-up period (6 months) during which time participants may have lost interest or found it too demanding to take the supplements every day. One reason for the lower drop-out rate in the EMA-C study may have been because the baseline testing sessions (~3 hours versus ~5 hours) and follow-up period (6 weeks versus 6 months) were significantly shorter and less cognitively demanding than the OCEAN study. In the OCEAN study participants were required to take four capsules a day of the placebo or active supplements. Some participants found it difficult to remember to take this many capsules which could have contributed to non-compliance and potentially the high drop-out rate (although this was not explicitly stated). For the EMA-C study some participants did not like the taste of the medication. This may have influenced the higher drop-out rate in the placebo group; those who did not like the taste and were not deriving benefits may have been more likely to drop-out (although again this was not explicitly stated). Future longitudinal *n*-3 PUFA studies should employ a shorter follow-up period (~3-4 months) and testing sessions (~2-3 hours). The testing sessions should not be too cognitively demanding. Future studies of *n*-3 PUFA supplementation should consider the

dosage (perhaps using a higher concentration supplement and therefore fewer capsules) and of Sativex, the taste (try to mask an unpleasant taste) of the medication.

A small number of people were excluded who were considered to be cannabis-dependent. This criteria was in place due to the potential for abuse of Sativex at high doses (Schoedel et al., 2011), and to avoid prior potential effects of cannabis on ADHD, which remain unknown. Furthermore, participants were asked to abstain from cannabis use for 30 days prior to study entry; those who were cannabis dependent or used cannabis on a regular basis might experience withdrawal during this period. However previous research has found heavy cannabis users to have a more positive response to cannabis than occasional users (D'Souza et al., 2008; Ramaekers et al., 2009). It is therefore possible that those with ADHD who are heavy cannabis users may have an optimum response to Sativex. Although we included 'daily' users who used 4-5 days a week, this was only 4 participants. A future trial could consider recruiting a larger sample of adults with ADHD with some who are also heavy cannabis users, to clarify whether effects may be stronger in this subgroup.

7.7 Implications

7.7.1 Clinical implications

- **Omega-3 PUFA supplementation as a treatment for children with ADHD (Chapters 2 and 3):**

– Given the small effects of *n*-3 PUFA supplementation on ADHD symptoms and potentially EL, *n*-3 PUFA supplementation could be used as an adjunctive treatment in children with ADHD. It should be considered as a monotherapy only for those who do not wish to use pharmacological treatment. Patients should be advised that there is no evidence for effects on cognition.

- **Omega-3 PUFA supplementation as a treatment for adults with ADHD (Chapter 5):**

– The efficacy of *n*-3 PUFA supplementation on improving symptoms of ADHD, EL and cognitive impairments in adults with ADHD is currently unclear. This is particularly the case given that we failed to find baseline differences in *n*-3 PUFA levels of adults with ADHD compared to controls. Omega-3 PUFA supplementation cannot currently be recommended as an evidence based-treatment for *adults* with ADHD.

- **Reward sensitivity in ADHD (Chapter 5):**

- Results suggesting sensitivity to reward and fast presentation rate in adults with ADHD as well as children, emphasises the advantages of incorporating fast-paced activities and incentives into the environment of both children and adults with ADHD (such as competitive sports). Patients should be informed of this recommendation.

- **Sativex as a treatment for adults with ADHD (Chapter 6):**

- Although the EMA-C study has provided promising results for the effect of Sativex in the treatment of adult ADHD, a further, definitive trial is required in order to examine efficacy and the underlying mechanisms involved. We cannot advise the use of Sativex on the basis of this one small study. The use of medications derived from illegal drugs such as 'cannabis' should not be discounted. Patients should be informed of the distinction between these and 'black market' drugs.

- **General observations regarding the treatment of ADHD in adulthood (Chapters 5 and 6):**

- There is considerable room for improvement in the NHS in terms of the treatment of ADHD in adulthood. Improved training and increased funding to the service is required. Improvements in training may also reduce any potential unease that some doctors may have in diagnosing and treating the disorder.
- It is important that clinicians bear in mind the high rates of overlap between ASD and ADHD, and also ADHD and depression/anxiety.

7.7.2 Implications for the general population

- **Omega-3 PUFA supplementation and cognition (Chapter 2):**

- There is no evidence to suggest that *n*-3 PUFA supplementation improves cognitive performance in the general population. This should be taken into account by individuals who are considering whether to purchase these products, and regulators and producers when promoting these products.

7.7.3 Future directions

- **Reward sensitivity in adults with ADHD (Chapter 5):**

  - Results of reward/presentation rate sensitivity in adults with ADHD emphasises the need for further studies in order to rigorously evaluate the causal role of 'state regulation' factors in ADHD across the lifespan. The observation that children and adults with ADHD may function better in fast-paced/incentive driven environments

highlights the need for more research into the optimal environments for patients with ADHD. One way in which this has been done is in a qualitative study which I have been involved in, which interviewed patients with ADHD about 'the positive aspects of ADHD'. Analysis of this data will be starting soon and results could be used to advise patients on lifestyle interventions in order to improve function.

- **Emotional dysregulation in adults with ADHD (Chapter 5):**
  - We propose the PASAT-C could be a suitable frustration task in adults with ADHD. Further research could take advantage of this experimental paradigm in the investigation of emotional dysregulation in adults with ADHD.
- **Omega-3 PUFA supplementation in adults with ADHD (Chapter 5):**
  - Large cross-sectional studies examining differences in n-3 PUFA levels in adults with ADHD compared to controls are required to clarify whether this difference is also present in adults. Longitudinal studies which examine n-3 PUFA levels and ADHD symptoms from childhood to adulthood are then required to clarify whether there is a causal relationship between n-3 PUFA and ADHD symptoms. If deficiencies in *n*-3 PUFA in adults are established along with evidence of a causal relationship, these studies should be used to establish suitable doses of *n*-3 PUFA to be used in treatment studies for adults with ADHD.
  - In the future we will combine our data on ADHD symptoms and EL with RCT data from 60 adults with ADHD (conducted by Michael Rösler's group at Saarland University (Germany)). This will provide us with increased power to examine treatment effects on these measures.
  - In the future the EEG data which we have collected as part of this study will be analysed to examine any potential treatment effects, we expect EEG could be a more sensitive measure to treatment effects than cognitive performance.
  - A large RCT of *n*-3 PUFA supplementation in ~ 352 adults with ADHD is required to examine for small but significant effects, similar to those seen in children with ADHD. Such a study should employ a shorter follow-up period (~3-4 months) and testing sessions (~1-2 hours). The testing sessions should not be too cognitively demanding. If possible the study could use a higher concentration supplement so that participants would need to take fewer capsules per day (~2 instead of 4 capsules). Researchers

should consider over-recruiting or using a sequential trial design to ensure a fully powered trial is conducted.

  - It is important to note that although future studies in adults should consider employing a shorter (~3-4 month) follow-up period, more trials of varying length in adults with ADHD may be required to confirm whether efficacy (or lack of efficacy) of *n*-3 PUFA is uninfluenced by trial duration.

- **Sativex as a treatment for adults with ADHD (Chapter 6):**
  - A large RCT examining the effect of Sativex compared to a stimulant medication in adults with ADHD is warranted. This should include 150-200 participants (in line with the sample size calculations estimated from the effects on cognition in the EMA-C study (Chapter 6, Section 6.6), have a longer follow-up period (~ 3 months), and have the ADHD symptom of hyperactivity/impulsivity as the primary outcome. Recruitment of a higher number of participants into the placebo group should also be considered to limit any bias caused by non-random drop-out. Dosing guidelines for the medication should be altered based on the optimum dose found in the current trial: the schedule should increase to a maximum dose of ~8-9 sprays per day, but recommend an optimum dosage of ~3-5 sprays. If possible any unpleasant flavour of the medication should be masked.
  - Given that the two cannabinoids in Sativex: delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) have been found to have differential, and even opposing, effects it is important that their effects in adults with ADHD are researched separately.

- **Mechanisms of Sativex and *n*-3 PUFA (Chapters 3, 5 and 6):**
  - One of the proposed causal underpinnings of the effect of *n*-3 PUFA and Sativex on ADHD symptoms is through alterations in dopamine levels (Assisi et al., 2006; Haag, 2003; Hibbeln et al., 2006; Young & Conquer, 2005). However this has yet to be formally tested. Future longitudinal studies could examine the relationship between changes in dopamine levels and ADHD symptoms following treatment with *n*-3 PUFA supplementation and Sativex.

7.8 Concluding remarks

Through the meta-analytic strategy of combining previous research and through two novel RCTs, this thesis has provided important insights into the effect of *n*-3 PUFA and the cannabinoid medication, Sativex, on cognitive performance, ADHD symptoms, and EL. This is in light of the evidence I have provided which shows adults with ADHD to be significantly impaired in these domains. I have found a generally null effect of *n*-3 PUFA on cognitive performance in both the general population, children with ADHD and related neurodevelopmental disorders, and in adults with ADHD. I have presented evidence to suggest there could be a small effect of *n*-3 PUFA supplementation in reducing EL in children and potentially adults with ADHD. I have also shown that *n*-3 PUFA could have a moderate effect on reducing symptoms of ADHD in adults which, combined with previous meta-analytic results, suggests that further investigation of *n*-3 PUFA as a treatment for adults with ADHD is warranted. I have finally provided evidence to suggest that the cannabinoid medication, Sativex, has shown promise at reducing the symptoms and cognitive impairments in adults with ADHD, which points strongly to the need for a future, larger treatment trial. The main aim of this thesis, to identify potential alternative treatments for adults with ADHD has therefore been met, with cannabinoid medication proving to be the most worthwhile area for future research. Omega-3 supplementation, whilst providing overall weaker evidence, has not yet been excluded as providing small to moderate effects similar to those seen in children with ADHD.

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Appendix A. Chapter 2 Supplementary files

Supplement 1

Table S1

Detailed breakdown of cognitive tasks included per study by the nine domains of cognition

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
TD				**Active**	**Placebo**
Antypa et al., (2009)	Inhibition	Go/No Go	Commission errors	27	27
	Attention (omission errors)	Go/No Go	Omission errors	27	27
	STM	15 words test	Delayed recall	27	27
		15 words test	Immediate recall	27	27
	MRT	Go/No Go	MRT	27	27
Baumgartner et al., (2012)	STM	HVLT	Total scores of recalls		
		HVLT	Recognition		
Benton et al., (2013)	MRT	Simple and choice RT	MRT	142	143
Dalton et al., (2009)	STM	HVLT	Total word recall	77	78
		HVLT	Word recognition	77	78
	Reading	Reading test (AGALa)	Reading	76	75
	Spelling	Spelling test (AGALb)	Spelling	76	75
Hamazaki et al., (1996)	Inhibition	Stroop test	Stroop score (%)	22	19
Jackson et al., (2012)	Inhibition	Stroop test	Stroop % accuracy	92	48
	WM	Numeric working memory	Accuracy (%)	92	48
		Alphabetic working memory	Accuracy (%)	92	48
		Three back task	Accuracy (%)	92	48
		Corsi blocks span	Span	92	48
	STM	Immediate word recall	Number correct responses	92	48

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
		Delayed word recall	Number correct responses	92	48
		Names to faces recall	Number correct responses	92	48
		Picture recognition	Accuracy (%)	92	48
		Word recognition	Accuracy (%)	92	48
		Telephone number task	Accuracy (%)	92	48
	MRT	Simple RT	RT	92	48
		Choice RT	RT	92	48
		Four choice RT	RT	92	48
Karr et al., (2012)	Inhibition	Stroop test	Interference	20	21
	STM	RAVLT	Word recall – average list 1-5	20	21
		RAVLT	Word recall after interference list 6	20	21
		RAVLT	Delayed word recall 7	20	21
Kennedy et al., (2009)	WM	Numeric working memory	Accuracy (%)	58	30
	STM	Immediate word recall[1]	% correct	55	30
		Paired associate learning	% error	55	30
		Spatial memory	Accuracy (%)	57	29
		Delayed word recall	Accuracy (%)	56	30
		Delayed picture recognition	% error	56	30
		Delayed word recognition	% error	53	30
	MRT	Simple reaction time	MRT	57	30
		Arrow RT	MRT	56	30
Kirby et al., (2010)	WM	Working memory test battery for children	Backwards digit recall	171	177
		Working memory test battery for children	Block recall	171	177
	STM	Working memory test battery for children	Digit recall	171	177
	Reading	WIAT-II	Word reading	171	177
		WIAT-II	Pseudoword reading	171	177
	Spelling	WIAT-II	Spelling	171	177
Mcnamara et al., (2010)	Inhibition	CPT	Commission errors	23	10

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
	MRT	CPT	MRT	23	10
Osendarp et al., (2007)	IQ	WISC-III, WAIS-III, NEPSY, WIAT	Combined: Design fluency, block design coding, vocabulary, digits backwards and mathematical reasoning	67	70
	STM	RAVLT	Combined: RAVLT-A3, RAVLT-slope, RAVLT delayed recall	67	70
Parletta et al., (2013)	Reading	WRAT-4	Word reading subtest	206	202
	Spelling	WRAT-4	Spelling subtest	206	202
Portillo-Reyes et al., (2014)	IQ	WISC-IV	Block design	30	20
		WISC-IV	Matrix reasoning	30	20
	Inhibition	Stroop color and word test	Stroop word		
		Stroop color and word test	Stroop color		
		Stroop color and word test	Stroop color-word		
	WM	WISC-IV	Letter number sequencing		
	STM	ENI	Verbal immediate recall		
		ENI	Verbal free recall		
		ENI	Verbal clue recall		
		ENI	Verbal recognition		
		ENI	Visual immediate recall		
	Reading	ENI	Comprehension instruction		
Stonehouse et al., (2013)	Inhibition	Stroop test	Interference	85	91
	WM	N-Back task, Corsi blocks span, Letter number sequencing	Total score	85	91
	STM	Immediate word recall, Delayed word recall, Delayed word recognition, Delayed picture recognition	Combined total (%)	85	91
	MRT	Choice reaction time task	MRT	85	91
ADHD+RD					
Gustafsson et al., (2010)	IQ	Ravens progressive matrices	Non-verbal reasoning	38	39
	Inhibition	QB Test	Impulsivity	37	33

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
	Attention (omission errors)	QB Test	Omission errors	37	33
Kairaluoma et al., (2009)	STM	WISC-III	Short-term verbal memory digit span	30	31
	Reading	Standardised reading test	Reading score	30	31
		Word reading	Accuracy (%)	30	31
		Pseudo word reading	Accuracy	30	31
		Text reading task	Accuracy	30	31
		Word reading	Speed	30	31
		Pseudo word reading	Speed	30	31
		Text reading	Speed	30	31
		ALLUa	Decoding fluency (raw points)	30	31
		NEPSY	Pseudoword reading (standardised score)	30	31
	Spelling	ALLUb	Spelling accuracy	30	31
Milte et al., (2012)	IQ	WISC-III	Vocabulary	57	30
	Reading	WIAT-III	Reading subtest	57	30
	Spelling	WIAT-III	Spelling subtest	57	30
	MRT	Go nogo	RT	40	23
Richardson and Montgomery, (2005)	Reading	WORD	Reading age	55	57
	Spelling	WORD	Spelling age	55	57
Richardson et al., (2012)	WM	BAS-II	Recall of digits backwards	180	182
	STM	BAS-II	Recall of digits forward	180	182
	Reading	BAS-II	Word reading achievement subtest: Standardised reading scores	180	182
		BAS-II	Word reading achievement subtest : Reading age	180	182
Sinn et al., (2008)	IQ	WISC III	IQ	45	38
	Inhibition	Stroop test	Stroop score	44	38
	WM	WISC III	Digits backwards	45	38

4

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
	STM	RAVLT	Digits forward	45	38
		RAVLT	Total recall (lists 1-5)	45	38
		RAVLT	Delayed recall	45	38
		RAVLT	20-min delayed recall	45	38
		RAVLT	Intrusions	45	38
		RAVLT	Recognition list A	45	38
Stevens et al., (2003)	Inhibition	CPT	Commission errors	18	13
	Attention (omission errors)	CPT	Omission errors	18	13
	MRT	CPT	RT	18	15
Vaisman et al., (2008)	Inhibition	TOVA	Commission errors	21	21
	Attention (omission errors)	TOVA	Omission errors	21	21
	MRT	TOVA	RT	21	21
	RTV	TOVA	RTV	21	21
Voigt et al., (2001)	Inhibition	TOVA	Commission errors	25	24
	Attention (omission errors)	TOVA	Omission errors	25	24
	MRT	TOVA	RT	25	24
	RTV	TOVA	RTV	25	24
Widenhorn-Müller et al., (2014)	WM	HAWIK-IV	Letter-number sequencing	37	41
		HAWIK-IV	Digits backward	29	32
	STM	HAWIK-IV	Digits forward	29	32
	Attention (omission errors)	KITAP/TAP	Go/No Go: omission errors	33	42

Reference	Domain(s) investigated[a]	Tasks[b]	Variables included in meta-analysis	Numbers in Analysis	
		KITAP	Sustained attention: omission errors	33	42
	MRT	KITAP/TAP	Go/No Go: reaction time	33	42
		KITAP	Sustained attention: reaction time	33	42

a. Where row is blank the domain is the same as above.
b. Where one study contributed multiple measures for a cognitive domain, a single SMD was derived from a meta-analysis of these assessments.
c. Exact N unknown and were estimated on a 1:1 ratio of the supplied total N.

Note. AGALa: Handleiding en Toets: Afrikaanse Groepleestoets vir Afrikaanssprekende Leerlinge in Graad 1/Sub A (Laubscher and Roux, 1984); AGALb: Handleiding en Toets: Afrikaanse Groepspellingtoets vir Afrikaans- sprekende Leerlinge in Graad 1/Sub A (Roux, 1984); ALLUa: Ala-asteen lukutesti (Lindeman, 1998); ALLUb: Ala-asteen lukutesti (Häyrinen et al., 1999); Text reading task (Niilo Mäki Institute, 1992); CPT: Continuous Performance Task; ENI: Evaluación Neuropsicológica Infantil (Matute et al., 2007); HAWIK-IV: Hamburg-Weschler-Intelligenztest für Kinder (Petermann and Ulrike, 2010); HVLT: Hopkins Verbal Learning Task (Brandt, 1991); NEPSY: Developmental Neuropsychological Assessment (Korkman et al., 1998); RAVLT: Rey Auditory Verbal Learning Test (Lezak, 1995); TOVA: Test of variables of attention (Greenberg and Kindschi, 1996); WAIS-III: Wechsler Adult Intelligence Scale (Wechsler, 1997); WIAT: Weschler Individual Achievement Test (Weschler, 1992); WIAT-II: Weschler Individual Achievement Test, Second edition (Wechsler, 2005); WIAT-III: Weschler Individual Achievement Test, Third edition (Wechsler, 2009); WISC-III: Wechsler Intelligence Scale for Children – Third edition (Weschler, 1991); WISC-IV: Weschler Intelligence Scales for Children – Fourth Edition (Weschler, 2007); WJ-R: Woodcock-Johnson Psycho-Educational Battery-Revised (Woodcock and Johnson, 1989); WORD: Weschler Objective Reading Dimensions (Wechsler, 1993); WRAT-4: Wide Range Achievement Test, Fourth Edition (Wilkinson and Roberts, 2006); KITAP: Test-batterie zur Aufmerksamkeitsprüfung für Kinder (Zimmermann et al., 2002); Stroop color and word test (Golden, 2005); TAP: Testbatterie zur Aufmerksamkeitsprüfung (Zimmermann and Fimm, 2009); TEA-ch: Test of Everyday Attention for Children (Manly et al., 1999); QB-Test: A continuous performance computerised test (Teicher et al., 1996); BAS-II: British Ability Scales Second Edition (Elliot et al., 1997); RTV: Reaction time variability; MRT: Mean Reaction Time; STM: Short-term memory; WM:Working memory

Table S2

Search strategy

Database	**Search Strategy**
Ovid Medline (1946-Sept week 2 2014) Embase (1974-2014 week 37) Psychinfo (1806-Sept week 3 2014)	Key word search: (“attention deficit hyperactivity disorder” OR “ADHD” OR “Cognition” OR “cognitive” OR "healthy") AND (“fish oils” OR “fatty acids” OR “omega 3 fatty acids” OR “docosahexaenoic acid” OR “DHA” OR “eicosapentaenoic acid” OR “EPA”) AND (“randomised controlled trial” OR “randomized controlled trial” OR “RCT”)
ClinicalTrials.gov	(“attention deficit hyperactivity disorder” OR “ADHD” OR “Cognition” OR “cognitive” OR "healthy") AND (“fish oils” OR “fatty acids” OR “omega 3 fatty acids” OR “docosahexaenoic acid” OR “DHA” OR “eicosapentaenoic acid” OR “EPA”)

Table S3

Study quality appraisal (scored as low, high or unclear risk)

Reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall risk	Other limitations
TD								
Antypa et al., (2009)	Low	Unclear	Low	Low	Low	Low	Unclear	No
Baumgartner et al., (2012)	Low	High	Low	Low	Low	Low	High	Yes
Benton et al., (2013)	Unclear	Unclear	Low	Low	Unclear	Unclear	Unclear	Yes
Dalton et al., (2009)	Low	High	High	Unclear	Low	Low	High	Yes
Hamazaki et al., (1996)	Unclear	Unclear	Low	Low	Low	Low	Unclear	Yes
Jackson et al., (2012)	Low	Low	Low	Low	Low	Low	Low	Yes
Karr et al., (2012)	Low	Low	Low	Low	Low	Low	Low	No
Kennedy et al., (2009)	Low	Low	Low	Low	Low	Low	Low	Yes
Kirby et al., (2010)	Unclear	Unclear	Low	Low	Unclear	Low	Unclear	Yes
Mcnamara et al., (2010)	Unclear	Unclear	Low	Low	Low	Low	Unclear	Yes
Osendarp et al., (2007)	Low	Low	Low	Low	Low	Unclear	Unclear	Yes
Parletta et al., (2013)	Low	Low	Low	Low	Low	Unclear	Unclear	Yes
Portillo-Reyes et al., (2014)	Unclear	Unclear	Low	Low	Unclear	Low	Unclear	Yes
Stonehouse et al., (2013)	Low	Low	Low	Low	Low	Low	Low	No
ADHD+RD								
Gustafsson et al., (2010)	Low	Low	Low	Low	Unclear	High	High	Yes

Reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall risk	Other limitations
Kairaluoma et al., (2009)	Low	Low	Low	Low	Low	Low	Low	Yes
Milte et al., (2012)	Low	Low	Unclear	Low	Low	Unclear	Unclear	Yes
Richardson and Montgomery, (2005)	Low	Low	Low	Low	Low	Low	Low	Yes
Richardson et al., (2012)	Low	Low	Low	Low	Low	Low	Low	Yes
Sinn et al., (2008)	Unclear	Low	Low	Low	Low	Low	Unclear	Yes
Stevens et al., (2003)	Unclear	Low	Low	Low	Low	Unclear	Unclear	Yes
Vaisman et al., (2008)	Low	Low	Low	Low	Low	Low	Low	Yes
Voigt et al., (2001)	Low	Unclear	Low	Low	Low	Low	Unclear	No
Widenhorn-Müller et al., (2014)	Low	Low	Low	Low	Low	Unclear	Unclear	Yes
Studies only included in qualitative synthesis: TD								
Long and Benton, (2013)	Low	Unclear	Low	Low	Low	Unclear	Unclear	Yes
Ryan and Nelson, (2008)	Low	Low	Low	Low	Unclear	High	High	Yes
Studies only included in qualitative synthesis: ADHD+RD								
Hirayama et al., (2004)	Low	Low	Low	Low	Low	High	High	Yes

Note. See Table S4 for a full description.

Table S4

Study quality appraisal

Reference	Reason if not low risk?	Other limitations
TD		
Antypa et al., (2009)	Allocation concealment: Allocation method was not specified.	Placebo group had significantly lower blood DHA concentration at baseline Olive oil placebo may have active properties
Baumgartner et al., (2012)	Allocation concealment: Once children were enrolled in the study they were assigned a group colour. All the tablet/capsule containers were in the respective colours and the children wore a name tag with the respective colour. Therefore although participants and researchers were blinded they were aware of the different groups children were in.	N-3 PUFA capsules were taken only 4 days per week. No assessment of blinding Does not state that capsules were identically flavoured Lack of generalisability: Population were iron deficient
Benton et al., (2013)	Random sequence generation/allocation concealment: Unspecified. Incomplete outcome data: Reason for drop-outs is unspecified. Selective reporting bias: Not all data (mean, SE) is presented (ie. one result only given in a figure)	One outcome was measured only at follow-up (no significant differences were found) Unclear whether placebo/active groups were similar at the start of the trial (participant demographics are not reported)
Dalton et al., (2009)	Blinding of participants and allocation concealment: *n*-3 PUFA were given to children as a spread. The active and placebo spreads were in different coloured containers therefore children and researchers may have been aware which participants were in different groups. Does not state that the placebo/active spreads were identically flavoured Blinding of outcome: The project leader was unblinded (single blind) although was not involved in any data collection or analyses.	Lack of generalisability: Children were of low socio-economic status with a very low fish intake. Supplements were given as a bread spread. No assessment of blinding was carried out.
Hamazaki et al., (1996)	Random sequence generation/Allocation concealment: Unspecified	No assessment of blinding
Jackson et al., (2012)	NA	No assessment of blinding. Does not state that capsules were identically flavoured, Olive oil placebo may have active properties
Karr et al., (2012)	NA	No assessment of blinding carried out. Capsules were not identically flavoured
Kennedy et al., (2009)	NA	No assessment of blinding carried out. Does not state that capsules were identically flavoured
Kirby et al., (2010)	Random sequence generation and allocation concealment: Unspecified Incomplete outcome data: High number of drop-outs	Supplements also contained vitamins (vitamin A, vitamin C, vitamin D, vitamin E)

Reference	Reason if not low risk?	Other limitations
	(N=71/450) reasons for drop-outs are not specified	No assessment of blinding, Olive oil placebo may have active properties
Mcnamara et al., (2010)	Random sequence generation: Method of randomisation not specified Allocation concealment: Allocation method was not specified	Lack of generalisability: Cognitive tasks completed during fMRI. No assessment of blinding was carried out
Osendarp et al., (2007)	Incomplete outcome data: Change scores for Indonesia sample are miscalculated meaning this group could not be included in the meta-analysis	Lack of generalisability: Supplement administered in a drink No assessment of blinding carried out.
Parletta et al., (2013)	Selective reporting bias: One scale (the Conners teacher rating scale) was unreported due to perceived unreliability as a result of high teacher/pupil turnover during the year (although these were obtained on request)	No assessment of blinding
Portillo-Reyes et al., (2014)	Random sequence generation and allocation concealment: Unspecified. Incomplete outcome data: Drop-outs occurred only in the placebo group (1 felt bad and 4 left school), although only 1 of these drop-outs may have related to the treatment.	An unequal number of participants were recruited: 25 placebo and 30 Active – 5 more were recruited in the active group to prevent drop-outs. Randomisation should have aimed for equal numbers across the active/placebo groups. No assessment of blinding. Does not state that capsules were identically flavoured. Lack of generalisability: Children were malnoursihed
Stonehouse et al., (2013)	NA	No assessment of blinding carried out. Does not state that capsules were identically flavoured
ADHD+RD		
Gustafsson et al., (2010)	Incomplete outcome data: 17 children dropped out because their parents wanted pharmacotherapy. It is unclear if this was equally distributed between placebo and active. Selective reporting bias: Results mainly reported for a subgroup of participants with a lower level of hyperactivity or higher level of oppositional behaviour. Data not shown for one cognitive task (QB test) - although all data was obtained on request	No assessment of blinding Does not state that capsules were identically flavoured
Kairaluoma et al., (2009)	NA	Supplements also contained Carnosine (an amino acid) No assessment of blinding. Does not state that supplements were identically flavoured.
Milte et al., (2012)	Blinding of participants: Overall 51% correctly guessed which group they were in. However 70% in	Does not state that capsules were identically flavoured.

Reference	Reason if not low risk?	Other limitations
	the active 2 group correctly guessed - above what would be expected by chance. Selective reporting: means and SDs are unreported for outcome measures, only treatment effects are reported although these were obtained on request	
Richardson and Montgomery, (2005)	NA	Supplements also contained evening primrose oil (20%) this is a bioactive substance with anti-inflammatory effects No assessment of blinding. Olive oil placebo may have active properties
Richardson et al., (2012)	NA	Blinding assessment carried out although only parents and teachers of participants were asked to guess treatment allocation
Sinn et al., (2008)	Random sequence generation: Unspecified	Supplements also contained evening primrose oil (600mg p/day) (as above) No assessment of blinding
Stevens et al., (2003)	Random sequence generation: procedure unspecified. Selective reporting: only change scores and not post-treatment means and SDs were reported although these were obtained on request	At baseline placebo and active groups differed significantly on reaction time in a cognitive task (CPT task), and on two parent-rated behaviour scales (the ASQ (abbreviated symptom questionnaire) and DBD (disruptive behaviour disorders rating scale)). No assessment of blinding. Does not state that capsules were identically flavoured. Olive oil placebo may have active properties. Re-calculation of effects from raw data obtained from the author indicated percentage change to have been mis-calculated
Vaisman et al., (2008)	NA	Supplements were given in a chocolate spread. Participants had to have a diagnosis of ADHD and impaired attention performance (a score lower than -1.8 (SD) from age and sex adjusted normal means Both may reduce generalisability of this studies findings No assessment of blinding
Voigt et al., (2001)	Allocation concealment: Unspecified	Does not state that capsules were identically flavoured
Widenhorn-Müller et al., (2014)	Selective reporting bias: Mean and SD not reported for 3 of the 4 cognitive tasks (although obtained on request)	Olive oil placebo may have active properties

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Reference	Reason if not low risk?	Other limitations
Studies only in qualitative synthesis: TD		
Long and Benton, (2013)	Allocation concealment: Unspecified Selective reporting bias: Demographics unreported at baseline, SDs for whole data set are unreported	Unclear as to whether capsules were identically flavoured. Olive oil placebo may have active properties
Ryan and Nelson, (2008)	Incomplete outcome data: Unequal number of drop-outs – N=11 in the active and 5 in the placebo group and reasons not given Selective reporting: No report of statistics for primary outcome measures, only a description of non-significance	No assessment of blinding. Unclear as to whether capsules were identically flavoured
Studies only in qualitative synthesis: ADHD+RD		
Hirayama et al., (2004)	Selective reporting bias: Only medians reported.	No assessment of blinding

Table S5

Studies excluded at full text stage with reasons (N=25)

Reason for exclusion	**Studies**
Failure to report placebo group	Fontani et al., (2005)
Supplemented with Omega-6 only	Aman et al., (1987); Arnold et al., (1989)
Supplemented with ALA only	Dubnov-Raz et al., (2014); Raz et al., (2009)
Unsuitable population	Rogers et al., (2008); Nilsson et al., (2012)
Unsuitable outcome	Bélanger et al., (2009); Bradbury et al., (2004); Dean et al., (2014); Gesch, (2002); Hamazaki et al., (1998, 1999, 2002); Itomura et al., (2005); Johnson et al., (2009); Manor et al., (2012); Perera et al., (2012); Richardson and Puri, (2002); Sawazaki et al., (1999); Sinn and Bryan, (2007); Young et al., (2005); Zaalberg et al., (2010)
Unsuitable population and outcome	Hamazaki et al., (2008); Vakhapova et al., (2011)

Table S6

Description of studies included in quantitative and qualitative synthesis

Reference Disorder (ADHD+RD only) Country	Mean age, years (% male) Meds – ADHD+RD only	Supplements (dose/day)			Study duration	Design (% completed)	Domain(s) investigated	Numbers recruited	
TD		**Active 1**	**Active 2[a]**	**Placebo**				**Active**	**Placebo**
Antypa et al., (2009) Netherlands	22.4 (19)	1740mg EPA, 250mg DHA	NA	Olive oil	4 weeks	Parallel (96%)	Inhibition, Attention (omission errors), STM, MRT	28	28
Baumgartner et al., (2012) South Africa	8.9 (50.9)	420mg DHA, 80mg EPA (only 4 days/week)	NA	Medium chain triglycerides	8.5 months 37 weeks	Parallel (90%)	Short-term memory	81	80
Benton et al., (2013) Wales	21.8 (female only)	400mg DHA	NA	Maize/soya oil	7.1 weeks	Parallel (93%)	MRT	152[c]	153
Dalton et al., (2009 South Africa	8.2 (46)	82.16mg EPA, 191.66mg DHA, 335.02mg ALA, 1567.36mg LA	NA	Bread flour	6 months (26 weeks)	Parallel (90%)	STM, Reading, Spelling	91	92
Hamazaki et al., (1996) Japan	20-30 (36)	200-240mg EPA 1500-1800mg DHA	NA	Soybean oil	3 months (13 weeks)	Parallel (96%)	Inhibition	27	26
Jackson et al., (2012) England	22·2 (33)	90mg EPA, 450mg DHA	300mg EPA 200mgDHA	1g Olive oil	12 weeks	Parallel (100%)	Inhibition, WM, STM, MRT	92	48
Karr et al., (2012) America	20.2 (29)	480mg DHA, 720mg EPA	NA	Coconut oil	4 weeks	Parallel (95%)	Inhibition, STM	20	21

Reference Disorder (ADHD+RD only) Country	Mean age, years (% male) Meds – ADHD+RD only	Supplements (dose/day)			Study duration	Design (% completed)	Domain(s) investigated	Numbers recruited	
Kennedy et al., (2009) England	10.9 (50)	400mg DHA, 8mg EPA, 592mg vegetable oil	1000mg DHA, 20mg EPA, 1500mg vegetable oil	2500mg vegetable oil	8 weeks	Parallel (98%)	WM, STM, MRT	58	30
Kirby et al., (2010) Wales	9.1 (unspecified)	56mg EPA 400mg DHA 800mg Vit A, 60mg Vit C, 5mg Vit D, 3mg Vit E	NA	Olive oil	16 weeks	Parallel (83%)	WM, STM, Reading, Spelling	208	214
Mcnamara et al., (2010) America	9.2 (100)	400mg/d DHA	1200 mg/d DHA	Corn oil	8 weeks	Parallel (87%)	Inhibition, MRT	26	12
Osendarp et al., (2007) Australia	8.7 (59)	Base powder (8g protein, 12g sugar, 4g maltodextrin) Plus 88mg DHA, 22mg EPA	NA	Base powder	52 weeks	Parallel (70%)	IQ, Short term memory	67	71
Parletta et al., (2013) Australia	8.3 (53)	558mg EPA, 174mg DHA, 60mg GLA, 10·8 mg vit E	NA	Palm oil	20 weeks (40 weeks cross over)	One-way cross-over (76%)	Reading, Spelling	206	202
Portillo-Reyes et al., (2014) Mexico	9.2 (42)	180mg DHA, 270mg EPA	NA	Soybean oil	3 months (13 weeks)	Parallel (91%)	IQ, Inhibition, working memory, Short-term memory, reading	30	25
Stonehouse et al., (2013) New Zealand	33.3 (36)	1160mg DHA, 170mg EPA	NA	Sunflower oil	6 months (26 weeks)	Parallel (77%)	Inhibition, WM, STM, MRT	115	113
ADHD+RD									
Gustafsson et al., (2010) Clinical ADHD Sweden	7-12 (Not specified) No meds	500mg EPA, 2·7mg DHA 10mg Vit E	NA	Rapeseed oil/medium chain triglyceride	15 weeks	Parallel 84%	IQ, Inhibition, Attention (omission errors)	46	46

Reference Disorder (ADHD+RD only) Country	Mean age, years (% male) Meds – ADHD+RD only	Supplements (dose/day)			Study duration	Design (% completed)	Domain(s) investigated	Numbers recruited	
				s					
Kairaluoma et al., (2009) Dyslexia Finland	10.6 (57) No meds	500mg EPA, 400mg Carnosine	NA	Triglycerides and cellulose	13 weeks	Parallel 100%	STM, Reading, Spelling	30	31
Milte et al., (2012) Clinical + ADHD symptoms Australia	8.9 (79) No meds	1109mg EPA 108mg DHA Vit E	264mg EPA 1032mg DHA Vit E	1467mg LA	4 months (17 weeks)	Parallel 80%	IQ, Reading, Spelling, MRT	30 (active 2 = 28)	29
Richardson and Montgomery, (2005) DCD England	8.8 (67) No meds	558mg EPA 174mg DHA 60mg ALA, 9·6mg Vit E	NA	Olive oil	3 months (13 weeks) (6 month cross-over)	Crossover 94%	Reading, Spelling	60	57
Richardson et al., (2012) underperforming in reading England	8.7 (53) No Meds	600mg DHA Placebo	NA	Corn/soybean oil	16 weeks	Parallel 99%	WM, STM, Reading	180	182
Sinn et al., (2008) ADHD symptoms Australia	9.4 (74) No meds	558mg EPA, 174mg DHA, 60mg GLA, 10·8mg Vit E	NA	Palm oil	15 weeks	Parallel 81%	IQ, Inhibition, WM, STM	45	38
Stevens et al., (2003)	9.8 (87)	80 mg EPA 480 mg DHA	NA	800mg Olive oil	16 weeks	Parallel 66%	Inhibition, Attention (omission errors), MRT	25	22

Reference Disorder (ADHD+RD only) Country	Mean age, years (% male) Meds – ADHD+RD only	Supplements (dose/day)			Study duration	Design (% completed)	Domain(s) investigated	Numbers recruited	
clinical ADHD America	79% medicated	40mg AA 96mg GLA 24mg vitamin E							
Vaisman et al., (2008) Clinical ADHD Israel	9.3 (75) No meds	153mg EPA 96mg DHA 25mg ALA	NA	742mg rapeseed oil	3 months (13 weeks)	Parallel 72%	Inhibition, Attention (omission errors), MRT, RTV	28	26
Voigt et al., (2001) Clinical ADHD America	9.3 (78) All taking stimulants	345mg DHA	NA	Unspecified	4 months (17 weeks)	Parallel 85%	Inhibition, Attention (omission errors), MRT, RTV	27	27
Widenhorn-Müller et al., (2014) Clinical ADHD Germany	8.9 (78%) Un-medicated	600mg EPA, 120mg DHA, 15mg Vit E	NA	Olive oil	16 weeks	Parallel 86%	WM, STM, Attention (omission errors), MRT	55	55
Studies only in qualitative synthesis: TD									
Long and Benton, (2013) TD Wales	20.9 (100)	672mg DHA	NA	Fatty acids	12 weeks	Parallel 87%	Inhibition	51	51
Ryan and Nelson, (2008)TD America	4.3 (53)	400mg DHA	NA	Sunflower oil	16 weeks	Parallel 87%	Inhibition, Attention (omission errors),	85	90
Studies only in qualitative synthesis: ADHD+RD									

Reference Disorder (ADHD+RD only) Country	Mean age, years (% male) Meds – ADHD+RD only	Supplements (dose/day)			Study duration	Design (% completed)	Domain(s) investigated	Numbers recruited	
Hirayama et al., (2004) Clinical ADHD Japan	10.8 (80) 15% medicated	100mg EPA, 515mg DHA	NA	Olive oil	8 weeks	Parallel 100%	Attention (omission errors), Inhibition	20	20

a. Where active 2 is shown it was combined with active 1 for the purpose of analysis. NA = there was no active 2 group. Not included = there was an active 2 (or 3) group but it was unsuitable for inclusion.
b. N for analyses purposes differs by task (see breakdown in Table S1).
c. Exact N unknown and was estimated on a 1:1 ratio of the supplied total N.
Note. MRT=Mean reaction time; RTV=Reaction time variability; STM=Short-term memory, WM=Working memory

Table S7

Characteristics of studies included in qualitative synthesis

Characteristic	**Total**	**TD**	**ADHD+RD**
N (studies)	27	16 (children = 9, adult = 7)	11 (all children)
N (participants)	3700	2593	1107
% Male[1]	50.3%	43%	66%
Completion rate	86.9%	85.6%	89.9%
Medication	NA	NA	8 unmedicated 1 fully medicated 2 partially medicated
	Total (wtmean)	**TD (wtmean)**	**ADHD+RD (wtmean)**
Age	NA	Children = 8.4 years Adult = 24.7 years	9.1 years
Trial duration	17.9 weeks	19.1 weeks	15.05 weeks
EPA (daily dose)	322.2mg	258.17mg	501.3mg
DHA (daily dose)	417.1mg	438.5mg	366.6mg
ALA (daily dose)	194.34mg	Dose = 335·02mg (1 study only)	48.9mg

Note. wtmean = weighted mean

1. Two studies did not have this data available (Gustafsson et al., 2010; Kirby et al., 2010)

Section S1

Detailed description of meta-analysis results

IQ

Five trials in 434 participants evaluated IQ. Omega-3 PUFA supplementation did not improve IQ (SMD = 0.14; 95% CI: -0.07 to 0.35, z = 1.30, p = 0.19) with no heterogeneity (x^2 = 5.06, I^2 = 20.9%, p = 0.28). In the ADHD+RD group (3 studies, N = 247) there was no treatment effect (SMD = 0.05; 95% CI: -0.21 to 0.32, z = 0.38, p = 0.71) and no heterogeneity (x^2 = 0.13, I^2 = 0.0%, p = 0.94). In the TD group (2 studies, children only, N=187) there was no treatment effect (SMD = 0.26; 95% CI: -0.28 to 0.80, z = 0.95, p = 0.34) with significant heterogeneity (x^2 = 4.12, I^2 = 75.8%, p = 0.04).

Inhibition

Twelve studies in 809 participants evaluated inhibition. Omega-3 PUFA supplementation did not improve inhibition (SMD = -0.04; 95% CI: -0.22 to 0.14, z = 0.41, p = 0.68) with no heterogeneity (x^2 = 17.95, I^2 = 38.7 %, p = 0.08). In the ADHD+RD group (5 studies, N=274) there was no treatment effect (SMD = -0.12; 95% CI: -0.**44** to 0.**20**, z = 0.**72**, p = 0.**47**) and no heterogeneity (x^2 = 7.00, I^2 =42.8%, p =0.**14**). In the TD group (7 studies, N=535) there was no treatment effect (SMD = 0.01; 95% CI: -0.20 to 0.23, z = 0.12, p = 0.90) and no heterogeneity (x^2 = 9.76, I^2 = 38.5%, p = 0.14).

TD – Adult and children separately

In the adult sample (5 studies, N =452) there was no treatment effect (SMD = -0.09; 95% CI: -0·27 to 0·10, z = 0.90, p = 0·37) and no heterogeneity (x^2 = 0.67, I^2 = 0.0%, p = 0.96). In the child sample (2 studies, N =83) there was no treatment effect (SMD = 0.13; 95% CI: -0.67 to 0.93, z = 0.32, p = 0.75) and significant heterogeneity (x^2 = 3.92, I^2 = 74.5%, p = 0.05)

Attention (omission errors)

Six studies in 321 participant's evaluated attention measured through omission errors. Omega-3 PUFA supplementation did not improve omission errors (SMD = -0.**13**; 95% CI: -0.**33** to 0.07, z = 1.**27** , p = 0.**20**) with no heterogeneity (x^2 = 1.09 , I^2 = 0.0%, p = 0.96). In the ADHD+RD group (5 studies, N=267) there was no treatment effect (SMD =-0.12 ; 95% CI: -0.**33** to 0.**10**, z = 1.08 , p = 0.**28**) and no heterogeneity (x^2

=1.01 , I^2 = 0.0%, p = 0.91). There was 1 study in adults in the TD group (N= 54) which failed to find an effect (SMD = -0.20, 95% CI: -0.73 to 0.34).

Memory (working memory)

Eight studies in 1308 participants evaluated working memory. Omega-3 PUFA supplementation did not improve working memory (SMD = 0.09; 95% CI: -0.008 to 0.18, z = 1.79, p = 0.07) with no heterogeneity (x^2 = 7.29, I^2 = 3.9%, p = 0.40). In the ADHD+RD group (3 studies, N=506) the treatment effect approached significance (SMD = 0.23; 95% CI: -0.001 to 0.46, z = 1.95, p = 0.05) with no heterogeneity (x^2 = 3.03, I^2 = 33.9%, p = 0.22). In the TD group (5 studies, N=802) there was no treatment effect (SMD = 0.04; 95% CI: -0.07 to 0.15, z = 0.65, p = 0.52) and no heterogeneity (x^2 = 2.00, I^2 = 0.0%, p = 0.74).

TD – Adult and children separately: In the adult sample (2 studies, N=316) there was no treatment effect (SMD = 0.09; 95% CI: -0.09 to 0.28, z = 0.98, p = 0.33) and no heterogeneity (x^2 = 0.32, I^2 = 0.0%, p = 0.57). In the child sample (3 studies, N =486) there was no treatment effect (SMD = 0.01; 95% CI: -0.13 to 0.14, z = 0.08, p = 0.94) and no heterogeneity (x^2 = 1.13, I^2 = 0.0%, p = 0.57).

Memory (short-term memory)

Fourteen studies in 1914 participants assessed short-term memory. Omega-3 PUFA supplementation did not improve short-term memory (SMD = 0.07; 95% CI: -0.01 to 0.15, z = 1.64, p = 0.10), with no heterogeneity (x^2 = 18.31, I^2 = 29.0%, p = 0.15). In the ADHD+RD group (4 studies, N=567) there was no treatment effect (SMD = 0.03; 95% CI: -0.10 to 0.16, z = 0.47, p = 0.64) and no heterogeneity (x^2 = 0.37, I^2 = 0.0%, p = 0.95). In the TD group (10 studies, 1347 participants) there was no treatment effect (SMD = 0.08; 95% CI: -0.03 to 0.19, z = 1.45, p = 0.15) and significant heterogeneity (x^2 = 17.66, I^2 = 49.0%, p = 0.04).

TD – Adult and children separately: In the adult sample (4 studies, N=411) there was no treatment effect (SMD = -0.004; 95% CI: -0.17 to 0.17, z = 0.05, p = 0.96) with no heterogeneity (x^2 = 4.23, I^2 = 29.1%, p = 0.24). In the child sample (6 studies, N=936) there was no treatment effect (SMD = 0.12; 95% CI: -0.01 to 0.26, z = 1.77, p = 0.08) with no heterogeneity (x^2 = 10.48, I^2 = 52.3%, p = 0.06)

Short-term memory using SD of change

Four studies (N=761) contained the SD of the change for the effect of *n* -3 PUFA on short-term memory. As found using the pre-test SD, *n* -3 PUFA supplementation did not improve STM (SMD = 0.04; 95% CI: -0.08 to 0.15, $z = 0.64$, $p = 0.52$), with no heterogeneity ($x^2 = 0.26$, $I^2 = 0.0\%$, $p = 0.97$).

Reading

Eight studies in 1579 participants evaluated reading. Omega-3 PUFA supplementation did not improve reading (SMD = 0.02; 95% CI: -0.06 to 0.09, $z = 0.38$, $p = 0.70$), with no heterogeneity ($x^2 = 5.33$, $I^2 = 0.0\%$, $p = 0.62$). In the ADHD+RD (4 studies, 622 participants) there was no treatment effect (SMD = 0.01; 95% CI: -0.09 to 0.12, $z = 0.26$, $p = 0.79$) and no heterogeneity ($x^2 = 2.64$, $I^2 = 0.0\%$, $p = 0.45$). In the TD group (children only, 4 studies, N=957) there was no treatment effect (SMD = 0.02; 95% CI: -0.09 to 0.12, $z = 0.28$, $p = 0.78$) and no heterogeneity ($x^2 = 2.69$, $I^2 = 0.0\%$, $p = 0.44$).

Spelling

Six studies in 1167 participants evaluated spelling. Omega-3PUFA supplementation did not improve spelling (SMD = 0.03; 95% CI: -0.09 to 0.15, $z = 0.52$, $p = 0.60$), with no heterogeneity ($x^2 = 5.26$, $I^2 = 5.0\%$, $p = 0.39$). In the ADHD+RD group (N = 3, 260 participants) there was no treatment effect (SMD = 0.03; 95% CI: -0.34 to 0.40, $z = 0.14$, $p = 0.89$), and no heterogeneity ($x^2 = 3.91$, $I^2 = 48.9\%$, $p = 0.14$). In the TD group (3 studies, children only, N=907) there was no treatment effect (SMD = 0.02; 95% CI: -0.11 to 0.15, $z = 0.32$, $p = 0.75$), and no heterogeneity ($x^2 = 1.26$, $I^2 = 0.0\%$, $p = 0.53$).

Reaction Time (mean reaction time)

Eleven studies in 1035 participants evaluated reaction time. Omega-3 PUFA supplementation did not improve reaction time (SMD = -0.002; 95% CI: -0.12 to 0.12, $z = 0.04$, $p = 0.97$), with no heterogeneity ($x^2 = 11.43$, $I^2 = 12.5\%$, $p = 0.33$). In the ADHD+RD group (5 studies, 260 participants) there was no treatment effect (SMD = 0.09; 95% CI: -0.13 to 0.30 , $z = 0.77$, $p = 0.44$) and no heterogeneity ($x^2 = 2.15$, $I^2 = 0.0\%$, $p = 0.71$). In the TD group (6 studies, 775 participants) there was no treatment effect (SMD = -0.05; 95% CI: -0.22 to 0.12, $z = 0.60$, $p = 0.55$) and no heterogeneity ($x^2 = 8.56$, $I^2 = 41.6\%$, $p = 0.13$).

TD – Adult and children separately: In the adult sample (4 studies, N=655) there was no treatment effect (SMD = -0.09; 95% CI: -0.33 to 0.15, $z = 0.72$, $p = 0.47$) and significant heterogeneity ($x^2 = 8.34$, $I^2 =$

64.0%, p = 0.04). In the child sample (2 studies, N=120) there was no treatment effect (SMD = -0.002; 95% CI: -0.29 to 0.29, z = 0.02, p = 0.99) and no heterogeneity (x^2 = 0.20, I^2 = 0.0%, p = 0.66).

Reaction time (reaction time variability)

Two studies in 91 ADHD+RD participants investigated reaction time variability (RTV). Omega-3 PUFA supplementation had no effect on RTV (SMD = 0.**29**; 95% CI: -0.**70** to 1.28, z = 0.**57**, p = 0.**57**), with significant heterogeneity (x^2 = 5.54, I^2 = 82.0 %, p = 0.02).

Table S8

Subgroup meta-analyses of those studies which: 1) strictly met inclusion criteria, 2) Included those with (probable) *n*-3 PUFA deficiency, 3) Were high quality), 4) Had more homogenous cognitive deficits or 5) Supplemented with > 100mg EPA

Domain	N studies	N participants	SMD	95% CI	Heterogeneity	
					P	I^2 (%)
1. Strict Inclusion						
IQ[b]	5	434	0.14	-0.07 to 0.35	0.28	20.9
Inhibition[b]	12	809	-0.04	-0.22 to 0.14	0.08	38.7
Attention (omission errors)[b]	6	321	-0.13	-0.33 to 0.07	0.96	0.0
Memory (working memory)	7	960	0.15	0.03 to 0.27*	0.65	0.0
Memory (short-term memory)	12	1505	0.07	-0.03 to 0.16	0.08	39.3
Reading	6	1170	0.06	-0.04 to 0.16	0.58	0.0
Spelling	4	758	0.06	-0.15 to 0.27	0.16	41.8
Reaction time (mean reaction time) [b]	11	1035	-0.002	-0.12 to 0.12	0.33	12.5
Reaction time (reaction time variability) [b]	2	91	0.29	-0.70 to 1.28	0.02*	82.0
2. PUFA deficient						
Inhibition	3	257	0.09	-0.39 to 0.57	0.02*	75.5
Memory (working memory)	2	226	0.18	-0.08 to 0.45	0.72	0.0
Memory (Short-term memory)	3	331	0.26	0.09 to 0.43**	0.26	25.1
Reading	2	201	0.21	-0.06 to 0.49	0.83	0.0
Reaction time (mean reaction time)	2	207	0.04	-0.82 to 0.89	0.03	79.3
3. High quality						
Inhibition	4	399	-0.03	-0.24 to 0.18	0.37	3.7
Memory (working memory)	4	766	0.09	-0.04 to 0.22	0.96	0.0
Memory (Short-term memory)	5	805	0.01	-0.10 to 0.12	0.35	9.8
Reading	2	173	0.09	-0.14 to 0.31	0.21	37.1
Reaction time (mean reaction time)	4	445	-0.02	-0.27 to 0.22	0.06	59.0
4. Cognitive impairment						
Memory (short-term memory)	2	285	0.15	-0.08 to 0.38	0.64	0.0
Reading	3	372	0.04	-0.11 to 0.19	0.28	21.7
Spelling	2	148	-0.17	-0.53 to 0.19	0.44	0.0
Reaction time (mean reaction time)	2	105	0.10	-0.29 to 0.49	0.60	0.0
5. Adequate EPA[c]						
IQ	4	297	0.19	-0.07 to 0.45	0.25	27.3
Inhibition	9	696	0.02	-0.17 to 0.22	0.09	41.8
Attention (omission errors)	4	241	-0.10	-0.33 to 0.12	0.92	0.0
Memory (working memory)	5	510	0.19	0.04 to 0.34*	0.47	0.0

Domain	N studies	N participants	SMD	95% CI	Heterogeneity	
					P	I^2 (%)
Memory (short-term memory)	8	666	0.06	-0.07 to 0.19	0.14	36.9
Reading	5	718	0.02	-0.10 to 0.14	0.58	0.0
Spelling	4	668	0.01	-0.20 to 0.22	0.23	29.7
Reaction time (mean reaction time)	6	550	-0.05	-0.25 to 0.15	0.11	44.1

a. Data was not available to meta-analyse for PUFA deficient for IQ, attention (omission errors), spelling and reaction time variability. Data for high quality studies was not available for IQ, attention (omission errors (1 study only)), spelling or reaction time variability (1 study only); data for 'without ALA' was not available for reaction time (reaction time variability), data for those who were cognitively impaired was not available for IQ, inhibition, reaction time (reaction time variability), attention (omission errors) and memory (working memory).
b. All studies met strict inclusion criteria therefore this analyses is the same as presented in Table 1 and Figure 2
c. This analysis could not be run for RTV as only one study supplemented with adequate EPA
*Significant at $p<.05$
**Significant after correction for multiple testing $p<.006$

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Appendix B. Chapter 3 Appendices

Table AB-1

Detailed breakdown of behavioural rating scales included in the meta-analysis per study by the six behavioural domains

First author	Domain(s) investigated	Rating scale	Measure included in meta-analysis[a]	Numbers in Analysis	
				Active	Placebo
ADHD+RND Group: ADHD only					
Gustafsson et al., (2010)	Oppositional behaviour – parent rated	CPRS	Oppositionality	46	46
	Oppositional behaviour – teacher rated	CTRS	Oppositionality	46	46
Manor et al., (2012)	Emotional lability – parent rated	CRS-P	Global emotional lability	94	41
	Oppositional behaviour – parent rated	CRS-P	Oppositional	99	42
	Emotional lability – teacher rated	CRS-T	Global emotional lability	93	41
	Oppositional behaviour – teacher rated	CRS-T	Oppositionality	92	40
Milte et al., (2012)	Emotional lability – parent rated	CPRS	Emotional lability	41	23
	Oppositional behaviour – parent rated	CPRS	Oppositionality	42	22
Sinn and Bryan, (2007)	Emotional lability – parent rated	CPRS	Global emotional lability	33	27
	Oppositional behaviour – parent rated	CPRS	Oppositionality	33	27

First author	Domain(s) investigated	Rating scale	Measure included in meta-analysis[a]	Numbers in Analysis	
				Active	Placebo
Stevens et al., (2003)	Oppositional behaviour – teacher rated	DBD	Oppositionality	20	12
	Oppositional behaviour – parent rated	DBD	Oppositionality	20	15
	Conduct problems – parent rated	DBD	Conduct	18	14
Widenhorn-Müller et al., (2014)	Oppositional behaviour – parent rated	CBCL	Delinquent behaviour	45	47
	Oppositional behaviour – teacher rated	CBCL - TRF	Delinquent behaviour	40	45
	Aggression	CBCL	Aggressive behaviour - parent rated	45	47
ADHD + RND Group: RND only					
Dean et al., (2014)	Aggression	CAS-parent	Total	12	7
	Aggression	MOAS-parent	Total	12	8
	Conduct problems – parent rated	SDQ	Conduct problems	12	7
Richardson and Montgomery, (2005)	Oppositional behaviour – teacher rated	CTRS	Oppositional	50	52
	Emotional lability –teacher rated	CTRS	Global emotional lability	50	52
Richardson et al., (2012)	Emotional lability – parent rated	CPRS	Emotional lability	180	182
	Oppositional behaviour – parent rated	CPRS	Oppositionality	180	182
	Emotional lability – teacher rated	CTRS	Emotional lability	180	182
	Oppositional behaviour – teacher rated	CTRS	Oppositionality	180	182

First author	Domain(s) investigated	Rating scale	Measure included in meta-analysis[a]	Numbers in Analysis	
				Active	Placebo
ADHD + RND Group: ADHD+RND					
Richardson and Puri, (2002)	Emotional lability – parent rated	CPRS	Emotional lability	15	14
	Oppositional behaviour – parent rated	CPRS	Oppositional	15	14

Note. SDQ = Strengths and Difficulties Questionnaire(Goodman, 1997); CPRS = Conners' Parent Rating Scales(Conners, 1990); CTRS = Conners' Teacher Rating Scales (Conners, 1990); CRS-T = Conners' Teacher Rating Scale Revised Long-Hebrew Version (Conners, 1998); CRS-P = Conners' Parent Rating Scale Revised Long – Hebrew Version (CRS-P) (Conners, 1998); DBD = Disruptive Behaviour Disorders (DBD) Rating Scale (Pelham, Gnagy, Greenslade, & Milich, 1992); CBCL = Child Behaviour Checklist (Arbeitsgruppe Deutsche Child Behavior Checklist, 1993a); CBCL-TRF = Child Behaviour Checklist – Teacher Report Form (Arbeitsgruppe Deutsche Child Behavior Checklist, 1993b); CAS – parent = The Children's Aggression Scale – Parent Version (Halperin et al., 2002); MOAS – parent = Modified Overt Aggression Scale – parent rated (Connor, 2002)

a. Where one study contributed multiple measures for a cognitive domain, a single SMD was derived from a meta-analysis of these assessments

Table AB-2

Search strategy

Database	**Search Strategy**
Ovid Medline (1946 to September week 3 2014) Embase (1974 to 2014 September 29th) Psychinfo (1806 to September week 4 2014)	Keyword search: ("attention deficit hyperactivity disorder" OR "ADHD" OR "emotional lability" OR "aggression" OR "oppositional" OR "conduct") AND ("fish oils" OR "fatty acids" OR "omega 3 fatty acids" OR "docosahexaenoic acid" OR "DHA" OR "eicosapentaenoic acid" OR "EPA") AND ("randomised controlled trial" OR "randomized controlled trial" OR "RCT")
Clinicaltrials.gov	("attention deficit hyperactivity disorder" OR "ADHD" OR "emotional lability" OR "aggression" OR "oppositional" OR "conduct") AND ("fish oils" OR "fatty acids" OR "omega 3 fatty acids" OR "docosahexaenoic acid" OR "DHA" OR "eicosapentaenoic acid" OR "EPA") AND ("randomised controlled trial" OR "randomized controlled trial" OR "RCT")

Table AB-3

Study quality appraisal (scored as low, high or unclear risk)

First Author	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Overall risk	Other limitations
ADHD+RND Group: ADHD only									
Gustafsson et al., (2010)	Low	Low	Low	Low	Unclear	Unclear	Low	Unclear	Yes
Manor et al., (2012)	Low	Low	Low	Low	Low	Low	Low	Low	Yes
Milte et al., (2012)	Low	Low	Unclear	Low	Low	Low	Low	Unclear	Yes
Sinn and Bryan, (2007)	Low	Unclear	Low	Low	Low	Low	Low	Unclear	Yes
Stevens et al., (2003)	Unclear	Low	Low	Low	Low	Low	Low	Unclear	Yes
Widenhorn-Müller et al., (2014)	Low	Low	Low	Low	Low	Unclear	Low	Unclear	Yes
ADHD+RND Group: RND only									
Dean et al., (2014)	Low	Low	Low	Low	Low	Low	Unclear	Unclear	Yes
Richardson and Montgomery, (2005)	Low	Low	Low	Low	Low	Low	Low	Low	Yes
Richardson et al., (2012)	Low	Low	Low	Low	Low	Low	Low	Low	Yes
ADHD+ RND Group: ADHD+RND									
Richardson and Puri, (2002)	Low	Low	Low	Low	Low	Low	Low	Low	Yes
Studies only in qualitative synthesis									
Bélanger et al., (2009)	Unclear	Unclear	Low	Low	Low	High	Low	High	Yes

First Author	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Overall risk	Other limitations
Hirayama et al., (2004)	Low	Low	Low	Low	Low	High	Low	High	Yes

Note. See Table S4 for a full description

Table AB-4

Study quality appraisal

First Author	Reason if not low risk?	Other limitations
ADHD+RND Group: ADHD only		
Gustafsson et al., (2010)	Incomplete outcome data: 17 children dropped out because their parents wanted pharmacotherapy. It is unclear if this was equally distributed between placebo and active. Selective reporting bias: Results mainly reported for a subgroup of participants with a lower level of hyperactivity or higher level of oppositional behaviour. Data not shown for one cognitive task (QB test).	No assessment of blinding Does not state that capsules were identically flavoured
Manor et al., (2012)	NA	Active supplements also contained phosphatidylserine (a phospholipid component). Does not state that capsules were identically flavoured.
Milte et al., (2012)	Blinding of participants: Overall 51% correctly guessed which group they were in. However 70% in the active 2 group correctly guessed - above what would be expected by chance	Does not state that capsules were identically flavoured.
Sinn and Bryan, (2007)	Allocation concealment: Unspecified	Supplements also contained evening primrose oil (600mg p/day) (as above) No assessment of blinding
Stevens et al., (2003)	Random sequence generation: procedure unspecified	At baseline placebo and active groups differed significantly on reaction time in a cognitive task (CPT task), and on two parent-rated behaviour scales (the ASQ (abbreviated symptom questionnaire) and DBD (disruptive behaviour disorders rating scale)). No assessment of blinding. Does not state that capsules were identically flavoured. Olive oil placebo

First Author	Reason if not low risk?	Other limitations
		may have active properties
Widenhorn-Müller et al., (2014)	Selective reporting bias: Mean and SD not reported for 2 of the 3 tested cognitive domains	Olive oil placebo may have active properties
ADHD + RND Group: RND only		
Dean et al., (2014)	Other bias: Small sample size (N=21)	Olive oil placebo may have active properties
Richardson and Montgomery, (2005)	NA	Supplements also contained evening primrose oil (20%) this is a bioactive substance with anti-inflammatory effects No assessment of blinding Olive oil placebo may have active properties
Richardson et al., (2012)	NA	Blinding assessment carried out although only parents and teachers of participants were asked to guess treatment allocation
ADHD + RND Group: ADHD+RND		
Richardson and Puri, (2002)	NA	Does not state that capsules were identically flavoured. Olive oil placebo may have active properties
Studies only in qualitative synthesis		
Bélanger et al., (2009)	Random sequence generation/allocation concealment: Unspecified Selective reporting bias: Two outcome measures are unreported	Does not state that capsules were identically flavoured.
Hirayama et al., (2004)	Selective reporting bias: Only medians reported.	No assessment of blinding Olive oil placebo may have active properties

Table AB-5

Studies excluded at full text stage with reasons (N=40)

Reason for exclusion	**Studies**
Failure to report placebo group	Fontani et al., (2005)
Unsuitable supplementation	Aman et al., (1987), Arnold et al., (1989), Raz et al., (2009), Dubnov-Raz et al., (2014)
Unsuitable population	Rogers et al., (2008), Hamazaki et al., (2008), Antypa et al., (2009), Benton et al., (2013), Hamazaki et al., (1998), Kirby et al., (2010), Long and Benton, (2013), Zaalberg et al., (2010), Itomura et al., (2005), Gesch et al., (2002),
Unsuitable outcome measures	Johnson et al., (2009), Kairaluoma et al., (2009), Perera et al., (2012), Sinn et al., (2008), Vaisman et al., (2008), Voigt et al., (2001), Young et al., (2005)
Unsuitable population and outcome measures	Nilsson et al., (2012), Vakhapova et al., (2011), Baumgartner et al., (2012), Bradbury et al., (2004), Dalton et al., (2009),Hamazaki et al., (2002, 1999, 1996), Jackson et al., (2012), Karr et al., (2012), Kennedy et al., (2009), Mcnamara et al., (2010), Osendarp et al., (2007), Parletta et al., (2013), Portillo-Reyes et al., (2014), Ryan and Nelson, (2008), Sawazaki et al., (1999), Stonehouse et al., (2013)

Table AB-6

Description of studies included in quantitative and qualitative synthesis

First Author Disorder Country	Mean age, years (% male) Meds	Supplements (dose/day)			Study duration	Design % completed	Domain(s) investigated	Numbers recruited	
		Active 1	Active 2[a]	Placebo				Active	Placebo
ADHD+RND Group: ADHD only									
Gustafsson et al., (2010) Clinical ADHD Sweden	7-12 (Unspecified but mixed) Unmedicated	500mg EPA, 2.7mg DHA 10mg Vit E	NA	Rapeseed oil/medium chain triglycerides	15 weeks	Parallel 84%	Oppositional behaviour (parent and teacher rated)	46	46
Manor et al., (2012) Clinical ADHD Israel	9.2 (71) Unmedicated	80mg EPA 40mg DHA 300mg PS	NA	Cellulose	15 weeks (15 week cross-over)	Cross-over 81%	Oppositional behaviour, emotional lability (parent and teacher rated)	99	60
Milte et al., (2012) Clinical + ADHD symptoms Australia	8.9 (79) Unmedicated	1109mg EPA 108mg DHA Vit E	264mg EPA 1032mg DHA Vit E	1467mg LA	4 months (17 weeks)	Parallel 80%	Oppositional behaviour, emotional lability (both parent rated)	30 (active 2 = 28)	29

First Author Disorder Country	Mean age, years (% male) Meds	Supplements (dose/day)			Study duration	Design % completed	Domain(s) investigated	Numbers recruited	
		Active 1	Active 2[a]	Placebo				Active	Placebo
Sinn and Bryan, (2007) ADHD symptoms Australia	9.4 (74) Unmedicated	558mg EPA, 174mg DHA, 60mg GLA, 10.8mg Vit E	NA	Palm oil	15 weeks	Parallel 81%	Oppositional behaviour, emotional lability (both parent rated)	45	38
Stevens et al., (2003) America Clinical ADHD	9.8 (87) 79% Medicated	80 mg EPA 480 mg DHA 40mg AA 96mg GLA 24mg vitamin E	NA	800mg Olive oil	16 weeks	Parallel 66%	Oppositional behaviour (parent and teacher rated), conduct problems (parent rated)	25	22
Widenhorn-Müller et al., (2014) Clinical ADHD Germany	8.9 (78%) Unmedicated	600mg EPA, 120mg DHA, 15mg Vit E	NA	Olive oil	16 weeks	Parallel 86%	Oppositional behaviour (parent and teacher rated), Aggression	46	49
ADHD+RND Group: RND only									
Dean et al., (2014) DBD[b] Australia	10.3 (81%) Unspecified	400mg EPA 2000mg DHA	NA	Low polyphenol olive oil, 10mg fish oil	6 weeks (6 week cross-over)	Cross-over 86%	Oppositional behaviour, conduct problems (both parent rated), aggression	12	9
Richardson and	8.8	558mg EPA	NA	Olive oil	13 weeks	Crossover	Oppositional	60	57

First Author Disorder Country	Mean age, years (% male) Meds	Supplements (dose/day)			Study duration	Design % completed	Domain(s) investigated	Numbers recruited	
		Active 1	Active 2[a]	Placebo				Active	Placebo
Montgomery, (2005) DCD England	(67) Unmedicated	174mg DHA 60mg ALA, 9.6mg Vit E			(13 week cross-over)	94%	behaviour, emotional lability (both teacher rated)		
Richardson et al., (2012) Under performing in reading England	8.7 (53) Unmedicated	600mg DHA	NA	Corn/soybean oil	16 weeks	Parallel 99%	Oppositional behaviour, emotional lability (parent and teacher rated)	180	182
ADHD+RND Group: ADHD+RND									
Richardson and Puri, (2002) LD+subclinical ADHD Ireland	10.25 (85) Unmedicated	186 mg EPA, 480mg DHA, 960mg LA, Vit E, 42 mg AA, 8mg thyme oil	NA	Olive oil	12 weeks	Parallel 78%	Oppositional behaviour, emotional lability (both parent rated)	22	19
Studies only in qualitative synthesis									
Bélanger et al., (2009) Clinical ADHD	9.18 (69) Unmedicated	500mg - 1000mg EPA (depending on body weight)	NA	500mg sunflower oil	8 weeks (8 week cross-	Cross-over 70%		19	18

First Author Disorder Country	Mean age, years (% male) Meds	Supplements (dose/day)			Study duration	Design % completed	Domain(s) investigated	Numbers recruited	
		Active 1	Active 2[a]	Placebo				Active	Placebo
Quebec					over)				
Hirayama et al., (2004) Clinical ADHD Japan	10.8 (80) 15% Medicated	100mg EPA, 514mg DHA	NA	Olive oil	8 weeks	Parallel 100%		20	20

a. Where active 2 is shown it was combined with active 1 for the purpose of analysis. NA = there was no active 2 group

b.76% met criteria for ADHD

Note. DBD = Disruptive Behaviour Disorder; LD = Specific learning difficulties; DCD = Developmental coordination disorder

Table AB-7

Characteristics of studies included in qualitative synthesis

Characteristic	**Frequencies**
N (studies)	12
N (participants)	1181
% Male[1]	68
Completion rate	88%
Medication	Unmedicated: 9 Medicated: 1 Partially medicated: 1 Unspecified: 1
	Weighted mean
Age (years)	9.1
Trial duration (weeks)	14.7
EPA (daily dose)	429.83mg
DHA (daily dose)	341.46mg
ALA (daily dose)	60mg[2]

1. One study did not specify sex ratio's and was therefore not included in this calculation(Gustafsson et al., 2010).

2. ALA was used in only one study(Richardson & Montgomery, 2005), therefore this is not the weighted mean.

Supplement AB-1

Detailed description of meta-analyses results

Emotional lability

Parent rated

Five trials in 650 ADHD+RND participants examined parent rated emotional lability (EL-P). There was no effect of *n*-3 PUFA on EL-P (SMD = 0.15; 95% CI: -0.07 to 0.36, z = 1.34, p = 0.18) with no heterogeneity (x^2 = 5.63, I^2 = 29%, p = 0.23).

Analysis using SD of the change

In the two trials(Manor et al., 2012; Richardson et al., 2012)manor that provided change score data there was no effect (SMD = 0.08; 95% CI: -0.41 to 0.57, z = 0.33, p = 0.74) with significant heterogeneity (x^2 = 5.42, I^2 =81.5%, p = 0.02).

Teacher rated

Three studies in 598 ADHD+RND participants examined teacher rated emotional lability (EL-T). There was no effect of *n*-3 PUFA on EL-T (SMD = 0.06; 95% CI: -0.19 to 0.31, z = 0.46, p = 0.65) with no evidence of heterogeneity (x^2 = 3.81, I^2 = 47.5%, p = 0.15).

Oppositional behaviour

Parent rated

Eight studies in 875 ADHD+RND participants investigated parent-rated oppositional behaviour (O-P). There was no effect of *n*-3 PUFA on O-P (SMD = 0.13; 95% CI: -0.01 to 0.27, z =1.77, p = 0.08) with no evidence of heterogeneity (x^2 = 7.45, I^2 =6.0%, p = 0.38).

Teacher rated

Six studies in 805 ADHD+RND participants investigated teacher rated oppositional behaviour (O-T). There was no effect of *n*-3 PUFA supplementation on O-T (SMD = 0.04; 95% CI:-0.10 to 0.18, z = 0.56, p = 0.57) with no evidence of heterogeneity (x^2 =4.08, I^2 =0.0%, p = 0.54).

Conduct problems

Parent rated

Two studies in ADHD+RND participants (N=51) examined parent rated conduct problems (C-P). There was no effect of *n*-3 PUFA on C-P (SMD = -0.22; 95% CI: -1.22 to 0.79, z =0.43, p = 0.67) with no evidence of heterogeneity (x^2 =2.86, I^2 =65.0%, p = 0.09).

Aggression

Two studies in 111 participants examined aggression. There was no effect of *n*-3 PUFA supplementation on aggression (SMD=-0.12; 95% CI: -0.47 to 0.22, z = 0.70, p = 0.48) and no heterogeneity (x^2 = 0.25, I^2 = 0.0%, p = 0.61).

Appendix C. Chapter 4 Appendices

Table AC-1

Adult Self Rating Scale for ADHD (ASRS) (Ronald C Kessler, Adler, Ames, et al., 2005)

Screening questionnaire

Please answer the questions below, rating yourself on each of the criteria shown using the scale on the right side of the page. As you answer each question, place an X in the box that best describes how you have felt and conducted yourself over the past 6 months.

		Never	Rarely	Sometimes	Often	Very often
1	How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?					
2	How often do you have difficulty getting things in order when you have to do a task that requires organization?					
3	How often do you have problems remembering appointments or obligations?					
4	When you have a task that requires a lot of thought, how often do you avoid or delay getting started?					
5	How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?					
6	How often do you feel overly active and compelled to do things, like you were driven by a motor?					
7	How often do you make careless mistakes when you have to work on a boring or difficult project?					
8	How often do you have difficulty keeping your attention when you are doing boring or repetitive work?					
9	How often do you have difficulty concentrating on what people say to you, even when they are speaking to you directly?					
10	How often do you misplace or have difficulty finding things at home or at work?					
11	How often are you distracted by activity or noise around you?					
12	How often do you leave your seat in meetings or other situations in which you are expected to remain seated?					
13	How often do you feel restless or fidgety?					
14	How often do you have difficulty unwinding and relaxing when you have time to yourself?					
15	How often do you find yourself talking too much when you are in social situations?					
16	When you're in a conversation, how often do you find yourself finishing the sentences of the people you are talking to, before they can finish them themselves?					
17	How often do you have difficulty waiting your turn in situations when turn taking is required?					
18	How often do you interrupt others when they are busy?					

Table AC-2

ADHD screening questionnaire: diagnostic criteria

If four or more marks appear in the darkly shaded boxes then the patient has symptoms highly consistent with ADHD in adults and further investigation is warranted.

		Never	Rarely	Sometimes	Often	Very often
1	How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?					
2	How often do you have difficulty getting things in order when you have to do a task that requires organization?					
3	How often do you have problems remembering appointments or obligations?					
4	When you have a task that requires a lot of thought, how often do you avoid or delay getting started?					
5	How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?					
6	How often do you feel overly active and compelled to do things, like you were driven by a motor?					

Table AC-3

Barkley childhood behaviour scale

Barkley Childhood Behaviour Scale – Self Report

Instructions

Please circle the number next to each item that best describes your behaviour when you were a child. PLEASE RATE YOUR BEHAVIOUR BETWEEN 7 and 12 YEARS OF AGE

Items:		Never or Rarely	Sometimes	Often	Very Often
1.	Failed to give close attention to details or made careless mistakes in my work	0	1	2	3
2.	Fidgeted with hands or feet or squirmed in seat	0	1	2	3
3.	Had difficulty sustaining my attention in tasks or fun activities	0	1	2	3
4.	Left my seat in classroom or other situations in which sitting was expected	0	1	2	3
5.	Didn't listen when spoken to directly	0	1	2	3
6.	Restless in the "squirmy" sense	0	1	2	3
7.	Didn't follow through on instructions and failed to finish work	0	1	2	3
8.	Had difficulty engaging in leisure activities or doing fun things quietly	0	1	2	3
9.	Had difficulty organising tasks and activities	0	1	2	3
10.	Felt "on the go" or acted as if "driven by a motor"	0	1	2	3
11.	Avoided, disliked, or was reluctant to engage in work that required sustained mental effort	0	1	2	3
12.	Talked excessively	0	1	2	3
13.	Lost things necessary for tasks or activities	0	1	2	3

14.	Blurted out answers before questions had been completed	0	1	2	3
15.	Easily distracted	0	1	2	3
16.	Had difficulty awaiting turn	0	1	2	3
17.	Forgetful in daily activities	0	1	2	3
18.	Interrupted or intruded on others	0	1	2	3

To what extent did the problems you may have circled on the previous page interfere with your ability to function in each of these areas of life activities **when you were a child between 7 and 12 years of age?**

	Areas:	Never or Rarely	Sometimes	Often	Very Often
1.	In your home life with your immediate family	0	1	2	3
2.	In your social interactions with other children	0	1	2	3
3.	In your activities or dealings in the community	0	1	2	3
4.	In school	0	1	2	3
5.	In sports, clubs, or other organisations	0	1	2	3
6.	In learning to take care of yourself	0	1	2	3
7.	In your play, leisure or recreational activities	0	1	2	3
8.	In your handling of your daily chores or other responsibilities	0	1	2	3

Table AC-4

Breakdown of exclusions OCEAN Study

Reason	**N**	**%**
Excluded (total n = 916)		**% of excluded**
Excluded due to mental health problems	**370**	**40.4**
Depression/Anxiety/panic disorder	46	5.0
Autism spectrum disorders (inc PDD)	211	23.0
OCD	34	3.7
Agoraphobia/Social phobia	6	0.7
Eating disorder	3	0.3
Psychosis/Schizophrenia	18	2.0
High suicidality	1	0.1
High risk: aggression/anger problems	5	0.5
Complex comorbidities	17	1.9
Bipolar	23	2.5
Tourettes syndrome	5	0.5
Chronic fatigue syndrome	1	0.1
Excluded due to substance abuse/dependence	**96**	**10.5**
Cannabis	52	5.7
Alcohol	19	2.1
Stimulants	1	0.1
Opiates	2	0.2
Complex/general substance abuse/dependence	22	2.4
Current psychoactive medication	**56**	**6.1**
Does not have ADHD on assessment/not enough symptoms	**167**	**18.2**
Physical health problems	**30**	**3.3**
Head injury/neurological problem/cognitive impairment (inc low IQ)	**107**	**11.7**
Other	**90**	**9.8**
Unsuitable age	51	5.6
Already taking Omega-3/6	6	0.7
Live too far away	6	0.7
In prison	5	0.5
Pregnant	4	0.4
Deceased	1	0.1
English not first language	3	0.3
Taking part in other research/treatment	14	1.5
Could not take part for other reasons (total n=619)		**% of could not take part for other reasons**
Declined	**163**	**26.3**
Not responded	**356**	**57.5**
Not enough information to screen	**86**	**13.9**
Booked to start study then dropped out	**14**	**2.3**

Note. PDD = Pervasive Developmental Disorder, OCD = Obsessive Compulsive Disorder, Participants with overlapping mental health/substance abuse exclusion categories: N=89. Headings in bold correspond to the exclusion reasons in Figure 4-1

Table AC-5

A comparison of ADHD symptom severity between those who were taking concomitant antidepressant medication and the remaining OCEAN sample (who were either unmedicated or medicated with stimulant or non-stimulant medication)

	Unmedicated/ ADHD meds (n=67)	**Concomitant Antidepressant medication (n=14)**	**t**	**p**
CW Inattention	27.36 (5.74)	26.21 (7.91)	0.63	0.53
CW Hyp/Imp	20.22 (6.11)	19.43 (4.07)	0.46	0.64
CW EL	17.69 (7.27)	19.43 (6.45)	-0.83	0.41

Table AC-6

Breakdown of exclusions EMA-C Study

Reason	**N**	**%**
Excluded (total n = 99)		**% of excluded sample**
Excluded due to mental health problems	**15**	**15.2**
Depression/Anxiety/panic disorder	1	1.0
Autism spectrum disorders	3	3.0
OCD	2	2.0
Psychosis/Schizophrenia	4	4.0
Tourettes	1	1.0
Complex comorbidities	1	1.0
Bipolar	3	3.0
Excluded due to substance abuse/dependence	**9**	**9.1**
Cannabis	4	4.0
Opiates	1	1.0
Complex/general substance abuse/dependence (or history)	4	4.0
Did not want to come off medication	**12**	**12.1**
Did not want to take cannabis	**5**	**5.1**
Current psychoactive medication	**11**	**11.1**
Does not have ADHD on assessment/not enough symptoms	**19**	**19.2**
Physical health problems	**7**	**7.1**
Epilepsy/seizures	**2**	**2.0**
Other	**19**	**19.2**
Religious reasons	1	1.0
Unsuitable age	3	3.0
Lived too far away	4	4.0
Work would not approve	4	4.0
Pregnant/breastfeeding	1	1.0
Could not take part as the study was fully recruited	1	1.0
Taking part in other research/treatment	1	1.0
Their psychiatrist did not approve them to take part	1	1.0
First degree relative with Psychosis/schizophrenia	3	3.0
Could not take part for other reasons (total n=104)		**% of could not take part for other reasons**
Declined	**18**	**17.3**
Not responded	**56**	**53.8**
More info needed/awaiting assessment	**25**	**24.0**
Booked to start study then dropped out	**5**	**4.8**

Note. OCD = Obsessive Compulsive Disorder.
Participants with overlapping mental health/substance abuse exclusion categories: N=13
Headings in bold correspond to the exclusion reasons in Figure 4-2

Supplement AC-1

Telephone screening for OCEAN study

Participant Checklist: Exclusion/Inclusion criteria to be checked over the telephone

****CONFIDENTIAL****

Question	**Guidance**	**Answer (Y/N) and notes**
1. Have you taken omega-3/6 supplements in the previous 6-months (exclude if they have taken regularly within past 3 months) (omega 3/6 = fish oils)		
2. Do you have any medical problems? (such as diabetes, thyroid problems or kidney disease)		
3. Do you have any known fish allergies?		
4. How much fish do you consume a week?		
5. Are you currently taking medication for your ADHD? (find out if it is slow release (they don't need to come off these for testing) eg. Atomoxetine or strattera or ask how many they take a day) • What medication are you taking and how many mg a day? • How long have you been taking this? (are they stable?)		
2. Have you EVER had any problems that have troubled you with regards to your mental health (apart from ADHD)? (this includes ASD and Aspergers?)		
3. Are you currently being treated for any other mental health problems? Including CBT (how long are they having this for), if psychotherapy/psychodynamic/counselling – ask how long for	Exclude if currently treated for OCD, psychosis, panic, anxiety disorder	
4. Are you currently taking any medication for mental health problems other than ADHD? If so, which medications and for what?	Exclude if on medication prescribed for a comorbid disorder apart from low dose of antidepressant/anti-anxiety meds	
5. Have you ever taken any medication for mental health problems other than your ADHD? If so, when were you last taking this medication?	Exclude if medicated within the 3 – months apart from if taking a low dose of antidepressant.	
6. Have you ever seen your own doctor about difficulties with nerves, tension, depression, anything related to your mental health (other than ADHD)? Did you get professional attention?		

Question	Guidance	Answer (Y/N) and notes
7. Have you ever had distinct episodes of depression or sadness different from what you're normally like lasting a week or more? If so, when was this? Yes? – Can you tell me about it? E.g symptoms, severity If no – go to Q 10	Exclude if currently experiencing a distinct episode of major depression. ie. more severe than usual.	
8. a) Have you had a period recently where you felt sad, miserable, depressed, empty or tearful most of the time? If yes: • When did you first feel like this? • Are you still feeling like this? • Have you felt like this for at least two weeks when NEARLY EVERY DAY, you felt sad, depressed, empty or tearful most of the time? b) Have you recently had a period of at least TWO WEEKS when, NEARLY EVERY DAY, you lost all interest in things, or got no pleasure from things which would usually make you happy? If yes: • When was this? • Are you still feeling like this?	If answer to a) is yes, try to gauge severity of depressed episode using additional questions. Exclude if currently experiencing a distinct episode of depression (yes to question b or severe enough on question a) If uncertain or yes to either/both questions follow up with more detailed questions.	
9. Have you had more than one spell like this in the last 2 years? I mean more than just one period when you have been seriously depressed or anxious	Consider excluding if depression is recurrent	
10. Have you had periods of feeling far more happy or energetic than your usual self, lasting for a week or more? So that your friends told you were talking too fast or that you were too 'hyper, compared to usual? If so, when was this?	Exclude if participant has experienced distinct episodes of mania/elation but not rapid cycling	
11. Do you drink alcohol?		
12. Approximately how many units of alcohol do you drink per day? Recommended limit for women 2-3 Units, for men 3-4 Units. **To calculate:** • Single shot is 1 unit • Alcopop is 2 Units • Can/pint of light beer is 1 unit. • Can/pint of lager is 2 Units • Can/pint of extra strong lager is 4 units • Party cocktail is 5 units Glass of wine (175ml) is 2 units	Exclude women who drink more than 6 units a day and men who drink more than 8 units.	
13. Do you smoke Cigarettes or Cigars?		
14. How many cigarettes or cigars do you smoke a day on average?		
15. Do you take any other drugs, legal or illegal? Which ones do you take? And how often do you take these?	Exclude if illegal drugs taken more often than twice	

Question	Guidance	Answer (Y/N) and notes
Do you take any other medications?	weekly – but not ecstasy/heroin	
16. Have you ever been addicted or dependent on any drugs or alcohol? If so, when was this?	Exclude if major history of drug or alcohol addiction, exclude if current substance abuse or addiction	
17. Have you ever suffered any injury to your head? If yes have you recovered from this/has it affected you in the long-term? Have you suffered from any neurological disorder (e.g. epilepsy, stroke, dementia)?	Exclude if they feel this has affected them in the long-term (eg. if Symptoms began from the injury) Exclude if answer to this question is yes	
18. Do you have any difficulty in reading? Are you dyslexic		
19. What is your first language?	Consider excluding if not English and if you do not think they can communicate effectively enough so as to not confound results on the reading test	

Follow up questions from question 7
ASK ONLY IF UNCERTAIN OR WANT TO GET BETTER PICTURE OF DEPRESSIVE STATE

During this time when you had worst two weeks where you felt sad, miserable or depressed

How was your appetite?	(check for weight loss/gain)	
How was your sleep pattern?	(check if slept too much or trouble falling asleep or erratic sleep pattern)	
Nearly every day, were you unable to make up your mind about things you ordinarily would have had no trouble deciding about?		
Did you lack in energy or feel much more tired than usual even if you had not been working very hard?		
Did you feel worthless nearly every day?		
Did you think a lot about your own death, or someone else's death or death in general?		

Supplement AC-2

Telephone screening for EMA-C study

Participant Checklist: Exclusion/Inclusion criteria to be checked over the telephone

Question	**Guidance**	**Answer (Y/N) and notes**
1. Do you have any physical health problems such as any problems with your liver, heart, kidneys		
2. Are you currently taking medication for your ADHD? • What medication are you taking and how many mg a day? • How long have you been taking this? (are they stable? ie. > 1 month)	Exclude if taking non-stimulant medication (Atomoxetine, Buproprion, Guanfacine or Clonidine)	
3. Do you take your medication every day? If not how many days a week do you take it?	Consider excluding if participant takes medication every day	
4. This trial requires you to come off your medication for 7 weeks (1 week before and the six weeks during the trial) Will that be a problem for you?	Only include if the patient (and psychiatrist) feels that coming off their medication will not represent a clinical problem in their overall control of symptoms and impairments	
5. Just to remind you this is a placebo controlled trial so there is a 50% chance that you may receive the placebo (dud medication that doesn't do anything) or the cannabis treatment – will that be okay?	Only include if patient feels comfortable with this	
6. Do you take cannabis currently? If so how often? Have you taken it in the past or are you planning on taking it in the next couple of months? Have you ever had any major problems after using cannabis? If yes, what problems have you had? Would you be able to come off cannabis for 30 days prior to starting the study	Exclude if currently cannabis dependent Consider excluding if previous adverse effects from cannabis (discuss with Philip) Exclude if unwilling to come off cannabis for 30 days prior to study entry	
7. As part of our protocol we can't give this medication to people who are pregnant or planning on becoming pregnant or currently breastfeeding. * Are you pregnant or breastfeeding or is your partner planning on becoming pregnant?	Exclude if pregnant or breastfeeding or if partner is planning on becoming pregnant.	

Question	Guidance	Answer (Y/N) and notes
5. Have you EVER had any problems that have troubled you with regards to your mental health (apart from ADHD)? Any depression or anxiety? (also includes ASD and Aspergers)	If yes for depression, move to questions 6, 7, 8, 9 to get a better picture of the health problem. If no to depression move to question 9	
6. Have you ever had distinct episodes of depression or sadness different from what you're normally like lasting a week or more? If so, when was this? Yes? – Can you tell me about it? E.g symptoms, severity If no – go to Q 9	Exclude if currently experiencing a distinct episode of major depression. ie. more severe than usual.	
7. a) Have you had a period recently where you felt sad, miserable, depressed, empty or tearful most of the time? If yes: • When did you first feel like this? • Are you still feeling like this? • Have you felt like this for at least two weeks when NEARLY EVERY DAY, you felt sad, depressed, empty or tearful most of the time? b) Have you recently had a period of at least TWO WEEKS when, NEARLY EVERY DAY, you lost all interest in things, or got no pleasure from things which would usually make you happy? If yes: • When was this? • Are you still feeling like this?	If answer to a) is yes, try to gauge severity of depressed episode using additional questions. Exclude if currently experiencing a distinct episode of depression (yes to question b or severe enough on question a) If uncertain or yes to either/both questions follow up with more detailed questions.	
8. Have you had more than one spell like this in the last 2 years? I mean more than just one period when you have been seriously depressed or anxious	Consider excluding if depression is recurrent	
9. Have you had periods of feeling far more happy or energetic than your usual self, lasting for a week or more? So that your friends told you you were talking too fast or that you were too 'hyper, compared to usual? If so, when was this? Does it cause problems for you/was this impairing?	Exclude if participant has experienced distinct episodes of mania/elation but not rapid cycling	
10. Are you currently being treated for any other mental health problems? Including CBT (how long are they having this for), if psychotherapy/psychodynamic/counseling – ask how long for	Exclude if currently treated for OCD, psychosis, panic, anxiety disorder	
11. Are you currently taking any medication for mental health problems other than ADHD? If so, which medications and for what?	Exclude if on medication prescribed for a	

Question	Guidance	Answer (Y/N) and notes
	comorbid disorder	
12. Have you ever taken any medication for mental health problems other than your ADHD? If so, when were you last taking this medication?	Exclude if medicated within the 3 – months	
13. Have you ever seen your own doctor about difficulties with nerves, tension, depression, any thing related to your mental health (other than ADHD)? Did you get professional attention?		
14. Do you drink alcohol?		
15. Approximately how many units of alcohol do you drink per day? Recommended limit for women 2-3 Units, for men 3-4 Units. **To calculate:** • Single shot is 1 unit • Alcopop is 2 Units • Can/pint of light beer is 1 unit. • Can/pint of lager is 2 Units • Can/pint of extra strong lager is 4 units • Party cocktail is 5 units Glass of wine (175ml) is 2 units	Exclude women who drink more than 6 Units a day and men who drink more than 8 Units.	
18. Do you take any other prescribed drugs? (as in for physical health problems) How often?	Exclude if psychiatrist considers drugs to be unsuitable for the study	
18. Do you take any other drugs legal or illegal? Which ones do you take? And how often do you take these? If no – are you planning to in the next few months? If yes – are you willing to not take these drugs during the 6 weeks of the study and for 1 week beforehand	Exclude if unwilling to stop taking drugs during the study. Exclude if taking drugs regularly	
18a. Have you ever been addicted or dependent on any drugs or alcohol? If so, when was this?	Exclude if major history of drug or alcohol addiction, exclude if current substance abuse or addiction and there is evidence of an ongoing problem	
19. Have you ever suffered any injury to your head? If yes have you recovered from this/has it affected you in the long-term? Have you suffered from any neurological disorder (e.g. epilepsy, stroke, dementia)?	Exclude if they feel this has affected them in the long-term (eg. if Symptoms began from the injury) Exclude if there were any serious brain problems as a result	
19. What is your first language?	Consider excluding if not English and if you do not think they can	

Question	Guidance	Answer (Y/N) and notes
	communicate effectively enough to not confound results	
20. Do you have any relatives with a diagnosis of Schizophrenia?	Exclude if parent, child or sibling has schizophrenia	

Follow up questions from question 5:

ASK ONLY IF UNCERTAIN OR WANT TO GET BETTER PICTURE OF **DEPRESSIVE** STATE

During this time when you had worst two weeks where you felt sad, miserable or depressed

How was your appetite?	(check for weight loss/gain)	
How was your sleep pattern?	(check if slept too much or trouble falling asleep or erratic sleep pattern)	
Nearly every day, were you unable to make up your mind about things you ordinarily would have had no trouble deciding about?		
Did you lack in energy or feel much more tired than usual even if you had not been working very hard?		
Did you feel worthless nearly every day?		
Did you think a lot about your own death, or someone else's death or death in general?		

CAARS-Observer-Report (18 item) and WRADDS

Participant ID_____ Date_____

'Please describe how much or how frequently each item describes you recently when you have not been taking your medication'

Use the scale 0-3: 0 = Not at all, never; 1 = Just a little, once in a while; 2 = Pretty much, often; and 3 = Very much, very frequently.

The following items will be read out loud to the participant and the participant will be asked to decide how much or how frequently each item describes them recently. Circle the number that corresponds to the participant's choice.

If more information is needed the following probes can be used:

Has this occurred in the last week?
Have others commented about this?
What have they said?
What difficulties or problems has this caused with other people or work?

	The person being described...	**Not at all, never**	**Just a little, once in a while**	**Pretty much, often**	**Very much, very frequently**
1	loses things necessary for tasks or activities (e.g. to-do lists, pencils, books, or tools).	0	1	2	3
2	talk too much.	0	1	2	3
3	gets rowdy or boisterous during leisure activities.	0	1	2	3
4	leaves seat when you are not supposed to.	0	1	2	3
5	has trouble waiting in line or taking turns with others.	0	1	2	3
6	has trouble keeping attention focused when working or at leisure.	0	1	2	3
7	is forgetful in daily activities.	0	1	2	3
8	has trouble listening to what other people are saying.	0	1	2	3
9	is always on the go.	0	1	2	3
10	fidgets (with hands or feet) or squirms in seat.	0	1	2	3
11	makes careless mistakes or has trouble paying close attention to details.	0	1	2	3
12	doesn't like academic studies/work projects where effort at thinking a lot is required.	0	1	2	3
13	is restless or overactive.	0	1	2	3
14	gives answers to questions before the questions have been completed.	0	1	2	3
15	has trouble finishing job tasks.	0	1	2	3
16	interrupts others when they are working or busy.	0	1	2	3
17	appears distracted when things are going on around him/her.	0	1	2	3
18	has problems organizing tasks and activities.	0	1	2	3

Appendix D. Chapter 5 Appendices

Table AD-1

Pearson correlation coefficient between commission errors (CPT, SART) and CW hyperactivity/impulsivity and omission errors and CW inattention in ADHD cases

	CW Hyp/Imp r(p)	**CW Inattention r(p)**
SART Commission errors	-0.01 (0.91)	-
SART Omission errors	-	0.03 (0.81)
CPT Commission errors	0.20 (0.10)	-
CPT Ommission errors	-	0.03 (0.78)

CW = CAARS/WRAADS investigator rating scale

Table AD-2

Pearson correlation coefficient between commission errors (CPT, SART) and CW hyperactivity/Impulsivity and omission errors and CW inattention in controls

	CW Hyp/Imp r(p)	**CW Inattention r(p)**
SART Commission errors	0.04 (0.82)	-
SART Omission errors	-	0.27 (0.15)
CPT Commission errors	0.19 (0.33)	-
CPT Ommission errors	-	-0.14 (0.45)

CW = CAARS/WRAADS investigator rating scale

Table AD-3

Spearman correlation coefficient PASAT, CNS-LS, ALS and CAARS/WRAADS emotional lability in ADHD cases

	CW EL r(p)	**CNS LS r(p)**	**ALS r(p)**
CNS LS	0.76 (<.0001)	-	-
ALS	0.67 (<.0001)	0.69 (<.0001)	-
PASAT Irritability post-task	0.29 (0.008)	0.17 (0.12)	0.08 (0.50)
PASAT Frustration post-task	0.45 (<.0001)	0.30 (0.006)	0.19 (0.10)

Table AD-4

Spearman correlation coefficient PASAT, CNS-LS, ALS and CAARS/WRAADS emotional lability in controls

	CW EL r(p)	**CNS LS r(p)**	**ALS r(p)**
CNS LS	0.67 (<.0001)	-	-
ALS	0.69 (<.0001)	0.53 (0.002)	-
PASAT Irritability post-task	0.02 (0.91)	0.14 (0.47)	-0.14 (0.46)
PASAT Frustration post-task	0.28 (0.14)	0.19 (0.30)	0.05 (0.81)

Table AD-5

Drop-outs by placebo and active group

Measure	**Active (n)**	**Placebo (n)**	**Chi²**	**Chi² (*p*)**	**Fisher's exact (*p*)**
Drop-out (total)	16	10	1.44	0.23	
Drop-out before T2	11	2	6.66	0.01**	
Drop out before T3	5	8	-	-	0.76
Drop-out reasons					
Lost to follow-up T2	7	2	-	0.31	1.00
Discontinued intervention T2	4	0	-	0.31	1.00
Lost to follow-up T3	1	8	-	-	0.007**
Discontinued intervention T3	4	0	-	-	0.007**

* Significant at $p \leq .05$

**Significant at $p \leq .01$

Table AD-6

Differences in ADHD symptoms between drop-outs and non-dropouts

	Drop-outs (n=26)	**Non drop-outs (n=55)**	***t***	**t-test (p)**
CW Inattention	26.31(6.96)	27.56(5.72)	0.86	0.39
CW Hyp/Imp	19.42(6.46)	20.40(5.50)	0.71	0.48
CW EL	16.77(7.71)	18.56(6.84)	1.06	0.29

Table AD-7

Breakdown of participant's medication status by placebo/active group

Medication	**Active (n)**	**Placebo (n)**
No meds	14	12
Stimulants	18	17
Non-stimulants	1	1
Stimulants and non-stimulants	2	1
Antidepressants only	2	1
Stimulants and anti-depressants	1	6
Non-stimulants and antidepressants	2	0
Stimulants and non-stimulants and antidepressants	1	0
Antidepressants and anti-anxiety medication	1	0
Stimulants and sleeping tablets	0	1

Table AD-8

Breakdown of the comorbid conditions between the placebo and active groups (rated using The MINI 6.0 (Mini International Neuropsychiatric Interview) diagnostic interview (Lecrubier et al., 1997)

Comorbid condition	**Active (N) (N=42)**	**Placebo (N) (N=39)**
Major depressive episode current	1	0
Major depressive episode past	20	15
Major depressive episode current and past	1	1
Major Depressive Episode Recurrent	11	8
Major Depressive Episode with Melancholic Features Current	0	0
Major Depressive Episode with Melancholic Features Past	10	8
Dysthymia Current (Past 2 years)	6	5
Suicidality Risk Current (Past month)	13	10
Level of Suicide Risk: Low	10	7
Level of Suicide Risk: Medium	1	2
Level of Suicide Risk: High	2	1
Hypomanic Episode (Current)	1	0
Hypomanic Episode (Past)	6	5
Manic Episode (Current)	1	0
Manic Episode (Past)	4	5
Panic Disorder (Lifetime)	8	3
Panic Disorder (Limited Symptom Attacks Lifetime)	8	11
Panic Disorder (Current)	3	0
Agoraphobia Current	5	4
Panic Disorder without Agoraphobia Current	1	1
Panic Disorder with Agoraphobia Current	2	0
Agoraphobia Current without History of Panic Disorder	1	2
Agoraphobia Current with History of Panic Disorder	3	2
Social Phobia Current (Past month)	6	3
Obsessive-Compulsive Disorder Current (Past month)	1	0
Post traumatic Stress Disorder Current (Past month)	2	0
Alcohol Dependence Past 12 Months	3	2
Alcohol Abuse Past 12 Months	0	3
Substance Abuse (Non alcohol) Past 12 Months	1	0
Substance Dependence (Non alcohol) Past 12 Months	2	2
Mood Disorder with Psychotic Features (Lifetime)	0	0
Mood Disorder with Psychotic Features (Current)	0	0
Psychotic Disorders (Current)	0	0
Psychotic Disorders (Lifetime)	0	0
Anorexia Nervosa Current (Past 3 Months)	0	0
Bulimia Nervosa Current (Past 3 Months)	1	0
Anorexia Nervosa, Binge Eating/Purging Type Current	0	0
Generalised Anxiety Disorder Current	11	5
Antisocial Personality Disorder Lifetime[a]	4	0

a. N= 41 active group

Table AD-9

Sensitivity analysis: Intent-to-treat analysis with multiple imputation (arbitrary imputation)

	Time x Treatment			
	Est	**SE**	***P***	**95% CI**
Primary outcome				
SART Commission errors	3.14	9.73	0.75	-17.79 to 24.07
SART Omission errors	3.15	9.49	0.74	-17.14 to 23.44
SART RTV	-12.51	44.97	0.79	-110.64 to 85.62
SART CV	1.15	7.08	0.87	-13.57 to 15.87
ADHD Symptoms				
CW Inattention	2.01	8.56	0.82	-15.86 to 19.88
CW Hyp/Imp	2.69	8.14	0.74	-14.12 to 19.49
CW EL	-0.32	12.77	0.98	-27.98 to 27.35
Cognition				
CPT Commission	4.65	13.26	0.73	-22.51 to 31.81
CPT Omission	9.48	16.58	0.57	-25.31 to 44.28
CPT RTV	9.15	14.85	0.54	-20.70 to 39.01
CPT CV	6.59	11.29	0.56	-16.12 to 29.30
Fast task MRT	24.21	36.75	0.52	-51.11 to 99.53
Fast task RTV	17.50	19.77	0.38	-22.31 to 57.31
Fast task reward MRT	3.47	19.40	0.86	-35.21 to 42.16
Fast task reward RTV	7.04	14.34	0.63	-22.41 to 36.49
Emotional lability				
CNS_LS	7.84	9.57	0.42	-11.74 to 27.42
ALS	4.41	10.94	0.69	-18.71 to 27.52
PASAT time to quit	0.22	16.86	0.99	-34.16 to 34.61
PASAT frustration pre-task	3.18	7.81	0.69	-13.07 to 19.42
PASAT frustration post-task	5.22	11.92	0.67	-20.28 to 30.73
PASAT Irritability pre-task	-0.88	7.17	0.90	-15.93 to 14.18
PASAT Irritability post-task	1.50	8.59	0.86	-16.37 to 19.37

Appendix E. Chapter 6 Appendices

Supplement AE-1: EMA-C Study: Dosing instructions (the below was transferred to diary format for the study)

First 14 days

- For the first 14 days, you should gradually increase the dose by one spray per day
- Please follow the dosing schedule according to the table below, up to a maximum of 14 sprays per day or until you find a dose that is effective
- **Do not exceed more than 14 sprays per day.**
- Please fill out Table 1 with the number of actual sprays you have used per day
- You should leave at least a 15 minute gap between sprays.
- **Spray the dose under your tongue or onto the inside of your cheek. Change the area in your mouth where you spray each time to avoid soreness.**

The remainder of the study

- After 14 days and for the remaining 28 days of the study please continue at the dose which you have found most effective.
- **Do not exceed more than 14 sprays per day.**
- Please spread the doses out throughout the day as best suits you
- Please leave at least a 15 minute gap between doses
- **Spray the dose under your tongue or onto the inside of your cheek. Change the area in your mouth where you spray each time to avoid soreness.**
- Please record the number of doses you spray each day in the note books that we have given you.
- Please keep the empty containers and bring them to your final research appointment

Table 1

Please follow and fill out this table for the first 2 weeks (transferred to diary format for the study with one page representing one day)

Day	**Number of sprays in the morning**	**Number of sprays in the afternoon and evening**	**Total number of sprays per day**	**Actual number of sprays**
1	1	0	1	
2	1	1	2	
3	1	2	3	
4	2	2	4	
5	2	3	5	
6	2	4	6	
7	3	4	7	
8	3	5	8	
9	3	6	9	
10	4	6	10	
11	4	7	11	
12	4	8	12	
13	5	8	13	
14	5	9	14	

Supplement AE-2: Safety monitoring and titration documents for the EMA-C study

General questions

1. Have you noticed any effects from the medication?

2. If you have noticed an effect how long do you think that effect has last for? (if an hour they may want to take it every hour)

Participant ID_____ Date_____

Adverse Events Scale

Please indicate below the frequency of any side effects experienced since the last medical appointment (mark with an X). Please contact your physician if side effects are significant.

SIDE EFFECT	**FREQUENCY**				
	Not at all 0	**Sometimes 1**	**Often 2**	**All the time 3**	**Comments**
Headache					
Dryness of the skin					
Dryness of the eyes					
Dryness of the mouth					
Thirst					
Sore throat					
Dizziness					
Nausea					
Stomach aches					
Vomiting					
Sweating					
Appetite reduction					
Weight loss					
Weight gain					
Diarrhea					
Frequent urination					
Tics					
Sleep difficulties					
Mood instability					
Irritability					
Agitation/excitability					
Sadness					
Heart palpitations					
Increased blood pressure					
Sexual dysfunction					
Feeling worse or different when the medication wears off (rebound)					
Paranoia					

Other (ask: are there any obvious side-effects):

CAARS-Observer (18 item)

Participant ID_____ Date_____

Observer name_____

Instructions: listed below are items concerning behaviours or problems experienced by adults. Read each item carefully and decide how much or how frequently each item describes this person in the **last 7 days**. Indicate your response for each item by circling the number that corresponds to your choice. Use the following scale: 0 = Not at all, never; 1 = Just a little, once in a while; 2 = Pretty much, often; and 3 = Very much, very frequently.

	The person being described...	**Not at all, never**	**Just a little, once in a while**	**Pretty much, often**	**Very much, very frequentl y**
1	loses things necessary for tasks or activities (e.g. to-do lists, pencils, books, or tools).				
2	talks too much.				
3	gets rowdy or boisterous during leisure activities.				
4	leaves seat when not supposed to.				
5	has trouble waiting in line or taking turns with others.				
6	has trouble keeping attention focused when working or at leisure.				
7	is forgetful in daily activities.				
8	has trouble listening to what other people are saying.				
9	is always on the go.				
10	fidgets (with hands or feet) or squirms in seat.				
11	makes careless mistakes or has trouble paying close attention to details.				
12	doesn't like academic studies/work projects where effort at thinking a lot is required.				
13	is restless or overactive.				
14	gives answers to questions before the questions have been completed.				
15	has trouble finishing job tasks.				
16	interrupts others when they are working or busy.				
17	appears distracted when things are going on around him/her.				
18	has problems organizing tasks and activities.				

Table AE-1: Comparison of drop-outs between active and placebo group for the per-protocol analysis

	Active (n=15)	**Placebo (n=15)**	**Fisher's exact (*p*)**
Drop-out (n)	1	4	0.33

Table AE-2: Intent to treat analysis (monotone imputation (MAR assumption))

	Time x treatment			
	Est	**SE**	***P***	**95% CI**
Primary outcome				
Qb	-0.46	1.15	0.69	-2.76 to 1.84
Post-hoc				
Qb Activity	0.07	1.17	0.95	-2.37 to 2.52
Qb Inattention	-0.33	1.20	0.79	-2.73 to 2.08
Qb Impulsivity	-0.14	1.59	0.93	-3.48 to 3.20
Secondary outcomes				
ADHD Symptoms				
CW Innattention	-2.72	1.56	0.08	-5.77 to 0.34
CW Hyp/Imp	-3.13	1.32	0.02	-5.74 to -0.51
CW EL	-0.18	1.38	0.90	-2.89 to 2.53
Cognition				
SART Commission errors	-2.13	2.55	0.40	-7.19 to 2.92
SART Omission errors	-0.38	5.23	0.94	-10.63 to 9.87
SART RTV	-3.90	6.64	0.56	-16.92 to 9.11
SART CV	0.81	3.18	0.80	-6.22 to 7.84
Emotional lability				
CNS_LS	-3.74	2.41	0.12	-8.47 to 0.98
ALS	-2.41	2.47	0.33	-7.28 to 2.46
Functional impairment				
WFIRS Total	0.08	1.26	0.95	-2.51 to 2.67

Table AE-3: Intent to treat analysis (arbitrary imputation (under MAR assumption))

	Time x Treatment			
	Est	**SE**	***P***	**95% CI**
Primary outcome				
Qb Test	0.25	1.45	0.87	-2.62 to 3.12
Post-hoc				
Qb Activity	-0.02	1.38	0.99	-2.80 to 2.75
Qb Inattention	-0.37	1.51	0.81	-3.39 to 2.65
Qb Impulsivity	-0.25	1.81	0.89	-3.95 to 3.46
Secondary outcomes				
ADHD Symptoms				
CW Innattention	-3.06	1.68	0.07	-6.37 to 0.26
CW Hyp/Imp	-3.08	1.70	0.08	-6.50 to 0.34
CW EL	-0.82	1.59	0.61	-3.95 to 2.31
Cognition				
SART Commission errors	-1.32	2.36	0.58	-5.96 to 3.33
SART Omission errors	-0.94	5.14	0.85	-11.01 to 9.13
SART RTV	-2.51	6.61	0.70	-15.46 to 10.44
SART CV	-0.19	1.94	0.92	-4.29 to 3.92
Emotional lability				
CNS-LS	-4.01	2.37	0.09	-8.66 to 0.65
ALS	-2.40	2.36	0.31	-7.06 to 2.25
Functional impairment				
WFIRS Total	-0.72	1.97	0.72	-4.91 to 3.47

Table AE-4: Intent-to-treat analysis (multiple imputation using the MNAR assumption)

	Time x Treatment			
Primary Outcome	**Est**	**SE**	**P**	**95% CI**
Qb Test	-0.72	0.99	0.47	-2.70 to 1.27
Post-hoc				
Qb activity	-0.11	1.28	0.93	-2.72 to 2.51
Qb inattention	-0.55	1.28	0.67	-3.14 to 2.04
Qb impulsivity	0.13	1.43	0.93	-2.76 to 3.03
Secondary outcomes				
ADHD Symptoms				
CW inattention	-2.72	1.71	0.11	-6.11 to 0.66
CW hyp/imp	-2.81	1.21	0.02	-5.19 to -0.44
CW EL	-0.67	1.53	0.66	-3.71 to 2.36
Cognition				
SART Commission errors	-1.78	2.26	0.43	-6.23 to 2.68
SART Omission errors	-0.58	5.23	0.91	-10.83 to 9.67
SART RTV	-2.71	6.71	0.69	-15.86 to 10.44
SART CV	-0.72	1.74	0.68	-4.47 to 3.02
Emotional lability				
CNS-LS	-3.48	2.27	0.13	-7.94 to 0.97
ALS	-2.88	2.28	0.21	-7.37 to 1.60
Functional impairment				
WFIRS Total	0.30	1.03	0.77	-1.89 to 2.50

Table AE-5: Feedback from participant's in the active group in response to the open question 'how has the medication made you feel overall' and why they guessed whether they were taking the placebo/active medication

Feedback (Each row represents one participant)
Tastes like cannabis, noticeable effects. Made them feel calm, clear and sometimes spacey, and more focussed when doing certain things. Quite liked it but would have liked to have more time to adjust to it or experimented more with doses
Noticed a considerable difference when sprayed, felt calm, relaxed and energetic, was able to do things that needed a higher level of concentration. Would like to take again if available, although slows you down a bit though.
Have felt effects, slightly stoned at times. Didn't help attention that much though. Slowed thoughts, sleeping better - would want to take it in the evening.
Didn't feel anything, tasted disgusting
Sustained periods of concentration which would have been difficult to achieve unmedicated. Effects were positive overall. Didn't like the taste, but no dry mouth or insomnia like she gets from usual ADHD meds. Slept well and woke rested - major benefit. Stayed on tasks for longer generally. Would prefer to take over ADHD meds.
Sedating effects, calming effect and taste. Effected the depth of sleep. Thinks it works but individual does are too high, doesn't think it impairs. Mouth ulcers - aggressive oil.
He just knows it was active. Felt better, calmer and happier. Felt relaxed, but that effects took longer to kick in than street cannabis. A bit sedative, would want higher THC. Possibly still street cannabis if given a choice
Mainly the taste and when trying a higher dose, felt a bit odd. Definitely calmer and less anxiety/panicky feelings in situations where they might normally feel that way. Calmer at night, easier transition to wake in the morning.

Feedback (Each row represents one participant)
Experienced strong effects similar to smoked cannabis. Flavour tasted of cannabis. Became more engaged in activities and work, felt relaxed and able to sustain interest. Steadied his thought processes. Would definitely take as a treatment. Although vulnerability to anxiety at times.
Just feels like it had an effect on me. Everything becomes more focused, clearer, thoughts are clearer. Also lessens anxiety I have from time to time. Less of a come down that ritain and effects probably last for 1 to 2 hours.
Feel calmer and more relaxed after spray. Partner also noticed improvements. Would definitely take as medication, would not need it all the time though he doesn't think
Made me feel tired/sleep/lethargic/slowed me up. Pressure at the back of the head. Clarity of vision/focus of vision. Felt something going through me. Headaches initially. Slept the best in years, felt much calmer. Appetite increased.
No improvement - maybe slightly improved productivity, felt a little calmer. Side effects - odd thoughts on 8+ sprays a day, feeling detached, couldn't be bothered, in my own world and sometimes forgetful
When taking spray at highest doses I felt high 'ish'. I could watch boring movies and I loved them - wouldn't usually be able to do this. If the dose is too high became restless, talkative, confused, trapped in own head, saying wrong things